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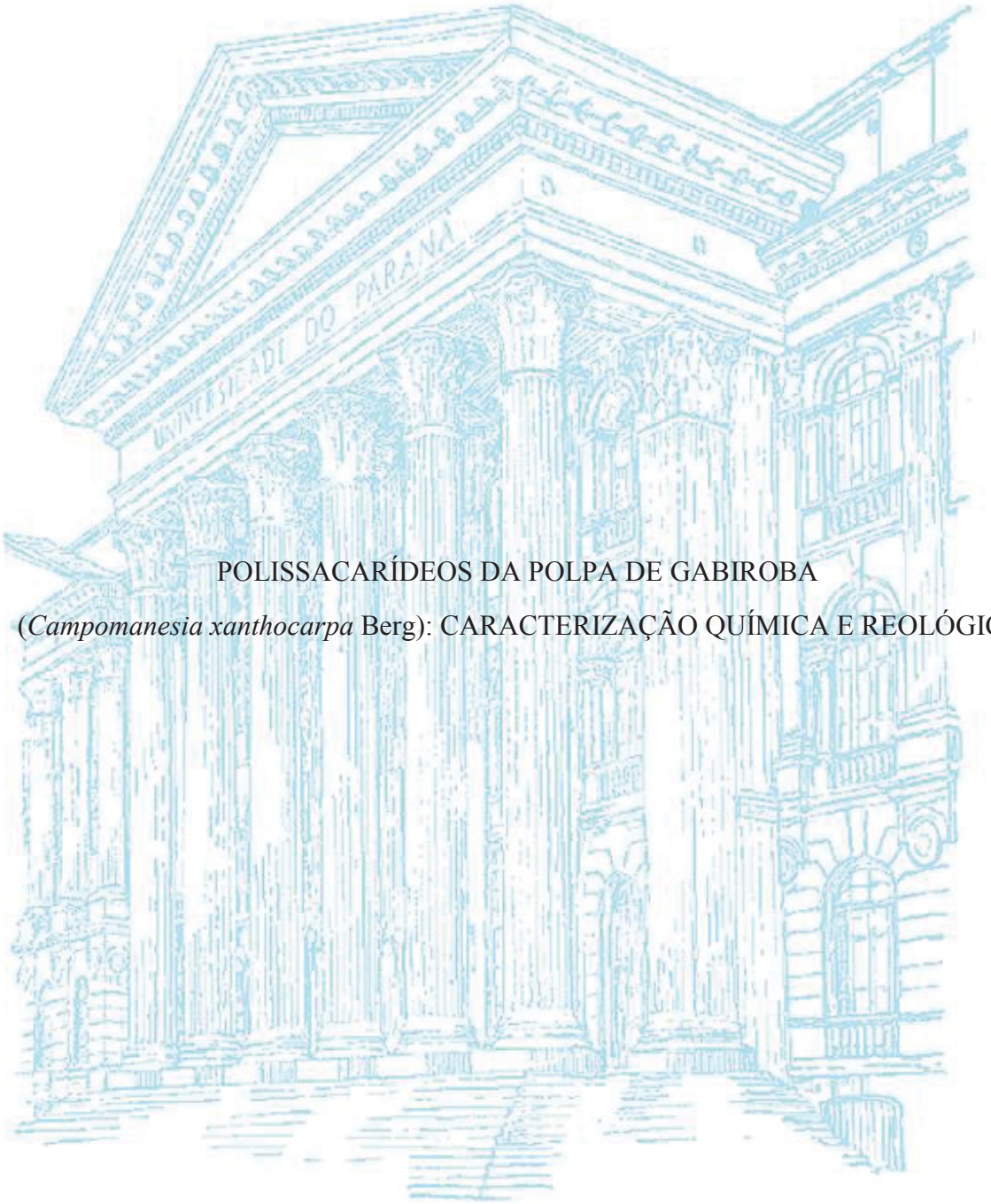

POLISSACARÍDEOS DA POLPA DE GABIROBA (*Campomanesia xanthocarpa* Berg): CARACTERIZAÇÃO QUÍMICA E REOLÓGICA

CURITIBA

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Tese apresentada como requisito parcial à obtenção do grau de Doutor em Ciências (Bioquímica), no Curso de Pós-Graduação em Ciências - Bioquímica, Setor de Ciências Biológicas, da Universidade Federal do Paraná - UFPR.

Orientadora: Profa. Dra. Joana Léa Meira Silveira
Coorientadoras: Profa. Dra. Carmen L. O. Petkowicz
Dra. Andrea Caroline Ruthes

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SHAYLA FERNANDA BARBIERI

POLISSACARÍDEOS DA POLPA DE GABIROBA (*Campomanesia xanthocarpa* BERG): CARACTERIZAÇÃO QUÍMICA E REOLÓGICA

Tese aprovada como requisito parcial para obtenção do grau de Doutor no curso de Pós-Graduação em Ciências-Bioquímica, Setor de Ciências Biológicas, Universidade Federal do Paraná, pela seguinte banca examinadora:

Prof.ª Dr.ª JOANA LÉA MEIRA SILVEIRA – Orientadora
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Prof.ª Dr.ª AGNES DE PAULA SCHEER
UFPR

Prof.ª Dr.ª TANIA MARI BELLE BRESOLIN
(UNIVALI)

Curitiba, 28 de fevereiro de 2018.

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RESUMO

O Brasil é um dos principais centros de diversidade genética de espécies frutíferas no mundo. No entanto, a maioria destas espécies ainda é pouco explorada do ponto de vista científico, como a *Campomanesia xanthocarpa* Berg (gabiroba). Assim, no presente trabalho, os polissacarídeos extraídos da polpa de gabiroba foram caracterizados estruturalmente. O comportamento reológico dos polissacarídeos pécticos, assim como da polpa (*in natura*) e de uma geleia desenvolvida com a polpa também foi analisado. Os polissacarídeos da polpa de gabiroba foram obtidos através de extrações sequenciais com água, ácido cítrico 5% e hidróxido de sódio 2 mol L^{-1} e 4 mol L^{-1}, e tiveram suas estruturas químicas finas caracterizadas pela primeira vez na literatura. Os polissacarídeos pécticos foram extraídos com água quente, e precipitados com etanol, obtendo-se a fração GW, a qual apresentou alto grau de metil-esterificação (DM de 60%). Na análise de composição monossacarídica, essa fração mostrou-se composta principalmente por arabinose (54.5%), ácido galacturônico (33.5%), galactose (7.6%) e ramnose (1.6%). Através das análises de RMN de ^{1}H e ^{13}C (1D e 2D) foi possível confirmar a presença de homogalacturonanas (HG) e ramnogalacturonanas (RG-I) na fração GW. Esta, foi submetida a processos de fracionamento por congelamento e degelo e tratamento com solução de Fehling obtendo-se a fração GWP-TEP, a qual apresentou-se composta majoritariamente por homogalacturonanas. A fração GW teve seu comportamento reológico analisado. Dessa forma, foram feitas soluções nas concentrações de pectina (GW) de 1%, 3% e 5%, na presença de NaCl 0,1 mol L^{-1}. Todas as soluções apresentaram comportamento pseudoplástico. Nas análises oscilatórias, a solução GW 1% apresentou comportamento de solução diluída, enquanto a solução a 3% apresentou-se como solução concentrada e a concentração de 5% teve comportamento típico de gel fraco. Em relação as hemiceluloses, através da extração com hidróxido de sódio 4 mol L^{-1}, foi possível isolar uma galactoglucomanana (GGM), descrita pela primeira vez para frutas da família Myrtaceae. Através da análise de HPSEC-MALLS-RI foi observado o perfil homogêneo da fração constituída pela GGM, com massa molar de 25.340 g mol^{-1}. A análise de composição monossacarídica mostrou que a GGM é composta por Man:Glc:Gal na proporção de 1:1:0.6. As análises de metilação e RMN sugerem que a GGM é formada por uma cadeia principal de unidades de β-D-Glc*p* e β-D-Man*p* (1→4) ligadas, com unidades de α-D-Gal*p* substituindo principalmente as unidades de β-D-Man*p* na posição O-6. A polpa de gabiroba (*in natura*) e a geleia formulada com a polpa foram investigadas quanto ao seu comportamento reológico. A polpa, analisada por microscopia (*SEM*), apresentou ultraestrutura heterogênea onde foi possível observar a presença de fibras que promovem um efeito de deslizamento quando a polpa é submetida ao cisalhamento durante as análises de comportamento de fluxo. Nas análises oscilatórias, a polpa apresentou comportamento de gel sendo termicamente estável. Assim como a fração péctica em solução, a geleia analisada apresentou comportamento pseudoplástico, descrito pelo modelo de Herschel-Bulkley. A geleia também mostrou um comportamento típico de gel nas análises oscilatórias, além de apresentar estabilidade, mantendo suas características reológicas após um período de armazenamento de 22 meses. Através do estudo das estruturas dos polissacarídeos obtidos da polpa de gabiroba, do comportamento reológico da fração péctica, bem como do comportamento reológico da polpa e da geleia, foi possível obter resultados que agregam no conhecimento desta fruta, bem como da família Myrtaceae, além de auxiliarem no direcionamento do seu uso em diferentes aplicações, contribuindo para a preservação desta espécie.

Palavras-chave: *Campomanesia xanthocarpa* Berg. Polpa de gabiroba. Myrtaceae Polissacarídeos. Pectinas. Hemiceluloses. Estrutura química. Reologia. Geleia de gabiroba.

ABSTRACT

Brazil is one of the main centers of genetic diversity of fruit species in the world. However, most of these species are still poorly explored from a scientific point of view, as *Campomanesia xanthocarpa* Berg (gabiroba). In the present work, the polysaccharides extracted from the gabiroba pulp had its chemical structures characterized. The rheological behavior of pectic polysaccharides, as well as the pulp, and a jam developed with the gabiroba pulp were analyzed. The polysaccharides present in the gabiroba pulp were obtained by sequential extractions with water, 5% citric acid, and 2 mol L^{-1} and 4 mol L^{-1} sodium hydroxide, and their chemical structures were characterized for the first time in the literature. The pectic polysaccharides were extracted with hot water and precipitated with ethanol to yield the fraction GW, which had high degree of methyl-esterification (DM 60%). The monosaccharide composition showed that this fraction is mainly composed of arabinose (54.5%) galacturonic acid (33.5%), galactose (7.6%) and rhamnose (1.6%). Through ^{1}H and ^{13}C NMR analyzes it was possible to confirm the presence of homogalacturonans (HG) and rhamnogalacturonans (RG-I) in the fraction GW that was subjected to freeze-thawing processes and fractionation with Fehling solution, giving rise to fraction GWP-PE, mainly composed of homogalacturonans. The rheological behavior of fraction GW, with highest yield, was investigated. Thus, pectin solutions were prepared at concentrations of 1%, 3% and 5% in the presence of 0.1 mol L^{-1} NaCl. All the solutions exhibited pseudoplastic behavior. In oscillatory analysis, 1% pectin solution showed liquid-like behavior, while solutions at 3% presented concentrated solution behavior and the concentration of 5% had typical weak gel behavior. Regarding the hemicellulose fractions, from the extraction with 4 mol L^{-1} sodium hydroxide, it was possible to isolate a galactoglucomannan (GGM), which was described for the first time for a fruit of the Myrtaceae family. By HPSEC-MALLS-RI analysis, it was possible to observe the homogeneous profile in the GGM, which had molar mass of 25,340 g mol^{-1}. Monosaccharide composition showed that GGM had Man:Glc:Gal in a molar ratio of 1:1:0.6. The NMR and methylation analysis suggested that the GGM was formed by a backbone of β-D-Glc*p* and β-D-Man*p* (1→4)-linked, with α-D-Gal*p* side chains mainly linked to the C-6 of β-D-Man*p* residues. The rheological behavior of the gabiroba pulp (*in nature*) and jam formulated with the pulp were investigated. Microscopy (SEM) showed the heterogeneous ultrastructure of the pulp, where the presence of fibers was observed. These fibers result in a slip effect when the pulp was subjected to shear in the flow behavior analysis. In oscillatory analyses, the pulp had a gel-like behavior thermally stable. As observed for GW, the jam showed shear-thinning behavior described by the Herschel-Bulkley model and a typical gel-like behavior in the oscillatory analysis. In addition, the jam showed good stability, maintaining its rheological characteristics after being stored for 22 months. The study on the structure of polysaccharides obtained from gabiroba pulp and the rheological behavior of GW fraction, as well as the rheological behavior of the pulp and jam, allow us to obtain information that contribute to the knowledge of this fruit and the Myrtaceae family. In addition, it was possible to suggest the use of the fruit for different applications, contributing to the preservation of this species.

Key-words: *Campomanesia xanthocarpa* Berg. Gabiroba pulp. Myrtaceae. Polysaccharides. Pectins. Hemicelluloses. Chemical structure. Rheology. Gabiroba jam.

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PECTINS FROM THE PULP OF GABIROBA (*Campomanesia xanthocarpa* Berg): STRUCTURAL CHARACTERIZATION AND RHEOLOGICAL BEHAVIOR

ARTIGO II

EXTRACTION, PURIFICATION AND STRUCTURAL CHARACTERIZATION OF A GALACTOGLUCOMANNAN FROM THE GABIROBA FRUIT (*Campomanesia xanthocarpa* Berg), MYRTACEAE FAMILY

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LISTA DE ABREVIATURAS, SIGLAS E SÍMBOLO

Solventes e reagentes

Ac_2O - Anidrido acético
$BaCO_3$ - Carbonato de bário
$CHCl_3$ - Clorofórmio
D_2O - Óxido de deutério
H_2SO_4 - Ácido sulfúrico
MeI - Iodeto de metila
Me_2SO - Dimetil sulfato
NaN_3 - Azida de sódio
$NaNO_2$ - Nitrito de sódio
NaOAc - Acetato de sódio
$NaBH_4$ - Borohidreto de sódio
TFA - Ácido trifluoroacético

Métodos analíticos

^{13}C-RMN - Ressonância magnética nuclear de carbono 13
^{1}H-RMN - Ressonância magnética nuclear de hidrogênio
COSY - Espectroscopia de correlação entre hidrogênios
DEPT - Intensificação sem distorção por transferência de polarização
GC-MS - Cromatografia em fase gasosa acoplada à espectrometria de massas
GLC - Cromatografia líquido-gasosa
HMBC - Espectroscopia de correlação heteronuclear de múltiplas ligações
HPSEC - Cromatografia de exclusão por tamanho de alta performance
HSQC - Espectroscopia de correlação heteronuclear *Single-Quantum*
MALLS - Espalhamento de luz laser com multiângulos
ppm - Partes por milhão
RI - Índice de refração
SEM - Microscopia eletrônica de varredura
TOCSY - Espectroscopia de correlação total entre hidrogênios

δ - Deslocamento químico

Termos associados à estrutura de polissacarídeos

AG - Arabinogalactana
AG-I - Arabinogalactana do tipo I
AG-II - Arabinogalactana do tipo II
DA - Grau de acetil-esterificação
DM - Grau de metil-esterificação
HG - Homogalacturonana
HM - Pectinas com alto grau de esterificação
LM - Pectinas com baixo grau de esterificação
M*w* - Massa molar ponderal média
RG - Ramnogalacturonana
RG-I - Ramnogalacturonana do tipo I
RG-II - Ramnogalacturonana do tipo II
UA - Ácido urônico

Termos associados a reologia

$\dot{\gamma}$ - Taxa de cisalhamento
G' - Módulo elástico ou módulo de armazenamento
G" - Módulo viscoso ou módulo de perda
K - Índice de consistência
n - Índice de comportamento de fluxo
Pa - Pascal
η - Viscosidade
τ - Tensão de cisalhamento

Frações polissacarídicas extraídas da polpa de gabiroba (C*ampomanesia xanthocarpa* Berg)

GW - Fração bruta de polissacarídeo obtido por extração aquosa a quente

GWS - Fração sobrenadante do congelamento e degelo, proveniente da fração bruta GW

GWP - Fração precipitada do congelamento e degelo, proveniente da fração bruta GW

GWP-FS - Fração sobrenadante obtida após tratamento com solução de Fehling, proveniente da fração GWP

GWP-FP - Fração sobrenadante obtida após tratamento com solução de Fehling, proveniente da fração GWP

GWP-TES - Fração sobrenadante obtida após tratamento com etanol acidificado e congelamento e degelo da fração GWP-FP

GWP-TEP - Fração precipitada obtida após tratamento com etanol acidificado e congelamento e degelo da fração GWP-FP

GCA - Fração bruta de polissacarídeo obtido por extração ácida

GA2M - Fração bruta de polissacarídeo obtido por extração com NaOH 2 mol L^{-1}

GA4M - Fração bruta de polissacarídeo obtido por extração com NaOH 4 mol L^{-1}

GA4MS - Fração sobrenadante do congelamento e degelo, proveniente da fração bruta GA4M

GA4MP - Fração precipitada do congelamento e degelo, proveniente da fração bruta GA4M

GA4MS-FS - Fração sobrenadante obtida após tratamento com solução de Fehling, proveniente da fração GA4MS

GA4MS-FP - Fração precipitada obtida após tratamento com solução de Fehling, proveniente da fração GA4MS

SUMÁRIO

NOTA EXPLICATIVA

Neste trabalho os resultados estão apresentados em formato de artigo científico de acordo com as normas do Programa de Pós-Graduação em Ciências – Bioquímica, da Universidade Federal do Paraná.

1 INTRODUÇÃO

As frutas são compostas principalmente por polissacarídeos, dentre os quais podemos destacar as hemiceluloses e as pectinas (CAFFALL; MOHNEN, 2009; BURTON; GIDLEY; FINCHER, 2010), que são de grande interesse pelas suas propriedades biológicas e potencial de aplicação nas áreas alimentícia, farmacêutica, cosmética, entre outras (MUNARIN; TANZI; PETRINI, 2012; NAQASH et al., 2017; NAIDU; HLANGOTHI; JOHN, 2018). Dessa forma, a produção industrial de pectinas movimenta o mercado global, sendo que no Brasil, em 2017, foram exportadas cerca de 6 mil toneladas de pectinas no valor de 76 milhões de dólares e importadas cerca de 482 toneladas no valor de 6 milhões de dólares, segundo o ministério de desenvolvimento, indústria e comércio exterior (ALICEWEB, 2018).

As espécies frutíferas nativas representam grande potencial econômico pela possibilidade de produção de frutas nutritivas e com sabor diferenciado, uma vez que o consumidor está sempre à procura de novos produtos (SARMENTO; SILVA; SILVA, 2012). No entanto, a maioria das frutas nativas brasileiras ainda é pouco explorada do ponto de vista científico, assim como a *Campomanesia xanthocarpa* Berg.

A *Campomanesia xanthocarpa* Berg (gabiroba) é conhecida popularmente pelas suas propriedades medicinais, atribuídas a diferentes partes da planta. Estas propriedades vêm sendo comprovadas cientificamente promovendo efeitos na redução de colesterol e glicemia (KLAFKE et al., 2010; VINAGRE et al., 2010; VIECILI et al., 2014), efeito antioxidante (SANTOS et al., 2012; SANTOS et al., 2013), anti-inflamatório (KLAFKE et al., 2016), protetor gástrico (MARKMAN; BACCHI; KATO, 2004) e antimicrobiano (CZAIKOSKI et al., 2015; PEREIRA et al., 2015).

A gabiroba também se destaca pelas suas propriedades nutricionais, principalmente pela grande quantidade de vitamina C (313 a 826 mg 100 g^{-1} em frutos maduros) (SANTOS et al., 2012; VALOR NUTRICIONAL DA GUABIROBA, 2015), que associadas com o aroma e sabor exótico fazem com que essa fruta seja atraente para o consumo como alimento *in natura*, além de ser uma matéria prima promissora para o uso em formulações alimentícias (LISBÔA; KINUPP; BARROS, 2011).

Devido à versatilidade de aplicações e considerando a escassez de estudos mais detalhados sobre os polissacarídeos de plantas nativas brasileiras, principalmente para espécies da família Myrtaceae, e o interesse na busca por estruturas de polissacarídeos com potencial de aplicação, o presente estudo, através do conhecimento da estrutura química fina e

das características reológicas dos polissacarídeos, da polpa *in natura* e da geleia formulada a partir da polpa, busca agregar valor ao fruto de gabiroba. O conhecimento das características estruturais e reológicas também contribuirá para o melhor aproveitamento desta matéria prima, buscando o desenvolvimento de produtos que poderão ser utilizados como fonte de renda alternativa para a agroindústria familiar e, dessa forma, estimular o cultivo da planta auxiliando na preservação desta espécie no Brasil.

1.1 OBJETIVOS

1.1.1 Objetivo Geral

Caracterizar a estrutura química dos polissacarídeos presentes na polpa de *Campomanesia xanthocarpa* Berg (gabiroba) e avaliar as propriedades reológicas das pectinas extraídas, da polpa de gabiroba *in natura* e da geleia formulada a partir da polpa.

1.1.2 Objetivos Específicos

- Extrair os polissacarídeos da polpa de *C. xanthocarpa*;
- Purificar as frações polissacarídicas de interesse, extraídas da polpa de *C. xanthocarpa*;
- Caracterizar a estrutura química dos polissacarídeos purificados provenientes da polpa de *C. xanthocarpa*;
- Avaliar as propriedades reológicas das pectinas extraídas da polpa de *C. xanthocarpa*;
- Avaliar as propriedades reológicas da polpa de *C. xanthocarpa* e de uma geleia produzida a partir da polpa.

2 REVISÃO DE LITERATURA

2.1 C*ampomanesia xanthocarpa* BERG

A comercialização de frutas desempenha um papel importante na economia, destacando-se o Brasil como terceiro maior produtor de frutas do mundo, atrás da China e da Índia (CLERICI; CARVALHO-SILVA, 2011). No Brasil são produzidas cerca de 45 milhões de toneladas de frutas por ano, das quais 65% são consumidas internamente e 35% destinam-se ao mercado externo. Em 2016, cerca de 31 mil toneladas de frutas foram exportadas para todo o mundo, no valor de aproximadamente 50 milhões de dólares, com o enfoque para a produção de conservas e formulações alimentícias (ABRAFRUTAS, 2017).

As árvores frutíferas nativas representam um grande potencial econômico, pela possibilidade de produção de frutas diferenciadas, saborosas e nutritivas, uma vez que o mercado consumidor está sempre à procura de novos produtos. No entanto, a maioria das frutas nativas brasileiras ainda é pouco explorada (SARMENTO; SILVA; SILVA, 2012), como é o caso de diferentes frutas pertencentes a família Myrtaceae.

A família Myrtaceae (BARROSO, 1978), compreende cerca de 145 gêneros e 5.970 espécies conhecidas até o momento (THE PLANT LIST - MYRTACEAE, 2013). Entre as principais frutas desta família destacam-se a goiaba (*Psidium guajava* L.), a jabuticaba (*Myrciaria jabuticaba*) e a pitanga (*Eugenia uniflora* L.), as quais já são comercializadas (FERREIRA et al., 2016). Porém, a maioria das frutas da família Myrtaceae ainda é pouco conhecida como o pessegueiro-do-mato (*Eugenia myrcianthes* Nied.), o cambuí (*Myrciaria tenella* Berg), o guamirim-cereja (*Eugenia florida*), a grumixama (*Eugenia brasiliensis*), a uvaia (*Eugenia uvalha* Cambess.), o araçá (*Psidium cattleianum*) e a gabiroba (*Campomanesia xanthocarpa* Berg), a qual foi objeto de estudo deste trabalho.

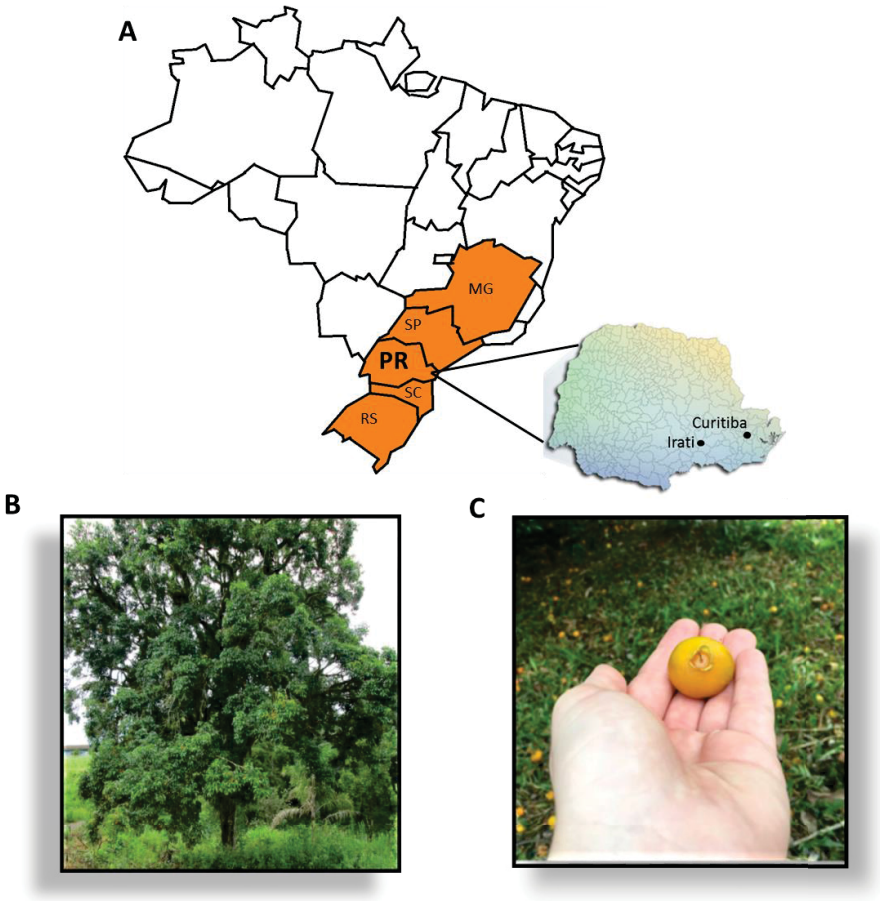
A *Campomanesia xanthocarpa* Berg é uma árvore frutífera nativa do Brasil (FIGURA 1B), encontrada principalmente dos estados de Minas Gerais ao Rio Grande do Sul (FIGURA 1A), sendo também relatada na literatura a sua presença em regiões do Paraguai, Bolívia e Argentina (LISBÔA; KINUPP; BARROS, 2011; SARMENTO; SILVA; SILVA, 2012).

A *Campomanesia xanthocarpa* apresenta como sinonímias botânicas: *C. crenata*, *C. dusenii*, *C. littoralis*, *C. malifolia* e *C. rhombea* e é conhecida popularmente como gabiroba, guabiroba, guavirova, guabiroba-miúda e guabiroba-do-mato dependendo da região em que é encontrada (SARMENTO; SILVA; SILVA, 2012). A floração desta planta normalmente

ocorre de setembro a novembro por um curto período de tempo, 10 a 15 dias, e a maturação dos frutos em 15 a 20 dias.

As frutas apresentam formato do tipo globoso de cor verde quando jovens e amarelos quando maduros, com cerca de 2,5 cm de comprimento e de 2-3 cm de largura, com epicarpo liso e fino (FIGURA 1C). O endocarpo é doce e suculento apresentando aroma cítrico intenso considerado agradável (RASEIRA et al., 2004; LISBÔA; KINUPP; BARROS, 2011). Recentemente, FERREIRA et al. (2016) extraíram e analisaram os compostos voláteis da polpa de gabiroba identificando 79 compostos como principais responsáveis pelo odor característico da fruta. Segundo os autores, esta informação é uma ferramenta potencial para o uso na avaliação de processamento e qualidade pós-colheita.

FIGURA 1 – *Campomanesia xanthocarpa* Berg (GABIROBA) (A) DISTRIBUIÇÃO GEOGRÁFICA DA GABIROBA NO BRASIL, DESTACANDO-SE NO ESTADO DO PARANÁ A CIDADE DE IRATI ONDE FORAM COLETADOS OS FRUTOS PARA ESTE ESTUDO. (B) ÁRVORE DE GABIROBA (C) FRUTO DA GABIROBA



FONTE: O autor (2018). Fotos: Carlos Amilcar de Carvalho Silva; Camila Silva Tamiello.

A fruta da gabiroba apresenta-se composta por 7% de cálice, 16% de sementes, 17% de casca, e 60% de polpa (SANTOS et al., 2012). Em relação à sua composição química, estudos mostram alto teor de água (79,1-83,5%), carboidratos totais (7,8-10,2%), fibras alimentares (4,1-9,8%), lipídios (0,7-1,9%), proteínas (1,0-1,1%) e compostos fenólicos (19,59 μg g^{-1}) (VALLILO et al., 2008; ANDRADE et al., 2012; SANTOS et al., 2012; VALOR NUTRICIONAL DA GUABIROBA, 2015). Além disso, pode-se destacar esta fruta como rica em vitamina C apresentando cerca de 313 a 826 mg 100 g^{-1} (SANTOS et al., 2012; VALOR NUTRICIONAL DA GUABIROBA, 2015), sendo o camu-camu (*Myrciaria dúbia*) a fruta que apresenta maior teor de vitamina C (2.010 mg 100 g^{-1}) (AKTER et al., 2011), seguido da acerola (*Malpighia emarginata*), com 1.074 mg 100 g^{-1} (VENDRAMINI; TRUGO, 2000), valores relacionados a massa das frutas frescas e maduras.

As propriedades nutricionais associadas com o aroma e sabor exótico fazem com que a gabiroba seja atraente para o consumo como alimento *in natura*, para a produção de suco e polpa congelada, além de ser uma matéria prima promissora para o uso em formulações alimentícias como doces, geleias e sorvetes, as quais vem sendo desenvolvidas por produtores rurais de forma artesanal (KINUPP; BARROS 2008; LISBÔA; KINUPP; BARROS, 2011).

A *Campomanesia xanthocarpa* também é conhecida popularmente pelas propriedades medicinais apresentadas por diferentes partes da planta, que através de estudos científicos vem sendo analisadas e comprovadas. Biavatti et al. (2004) observaram que o extrato aquoso obtido a partir da infusão das folhas de gabiroba induziu a redução no ganho de peso e a glicemia em ratos. O efeito relacionado a redução do índice glicêmico em ratos tratados com decocto das folhas também foi observado por Vinagre et al. (2010). Além disso, Viecili et al. (2014) e Klafke et al. (2010), mostraram o potencial de cápsulas, contendo folhas trituradas desta planta, em reduzir os níveis de colesterol sanguíneo em pacientes hipercolesterolêmicos. Estudos com extratos aquosos e hidroalcoólicos também apresentaram efeito antioxidante (SANTOS et al., 2012; SANTOS et al., 2013), anti-inflamatório (KLAFKE et al., 2016), e protetor gástrico (MARKMAN; BACCHI; KATO, 2004).

Em relação aos frutos, foram analisados os extratos etanólicos, os quais apresentaram efeito antioxidante e antimicrobiano, associados ao conteúdo de polifenólicos e carotenoides presentes nos extratos analisados (CZAIKOSKI et al., 2015; PEREIRA et al., 2015).

No contexto florestal, a gabiroba vem sendo gradativamente extinta do meio rural como resultado da substituição da mata nativa pelas plantações de pastagens. Em vista disso, a Empresa Brasileira de Pesquisa Agropecuária (Embrapa Florestas), através dos projetos de conservação da biodiversidade e valoração dos produtos da floresta com araucária (Rede

Conservabio e Conservabio II), iniciaram em 2008 estudos buscando informações sobre o nicho ecológico, composição genética e propriedades químicas de plantas nativas da região sul do Brasil.

Visando à conservação e o uso sustentável destas plantas, os projetos Conservabio e Conservabio II, foram desenvolvidos em parceria com as comunidades de agricultores da região de Irati-PR, as quais definiram algumas espécies frutíferas nativas como a pitanga (*Eugenia uniflora* L.), o araçá (*Psidium cattleianum*) e a gabiroba (*Campomanesia xanthocarpa* Berg), como espécies prioritárias para os estudos.

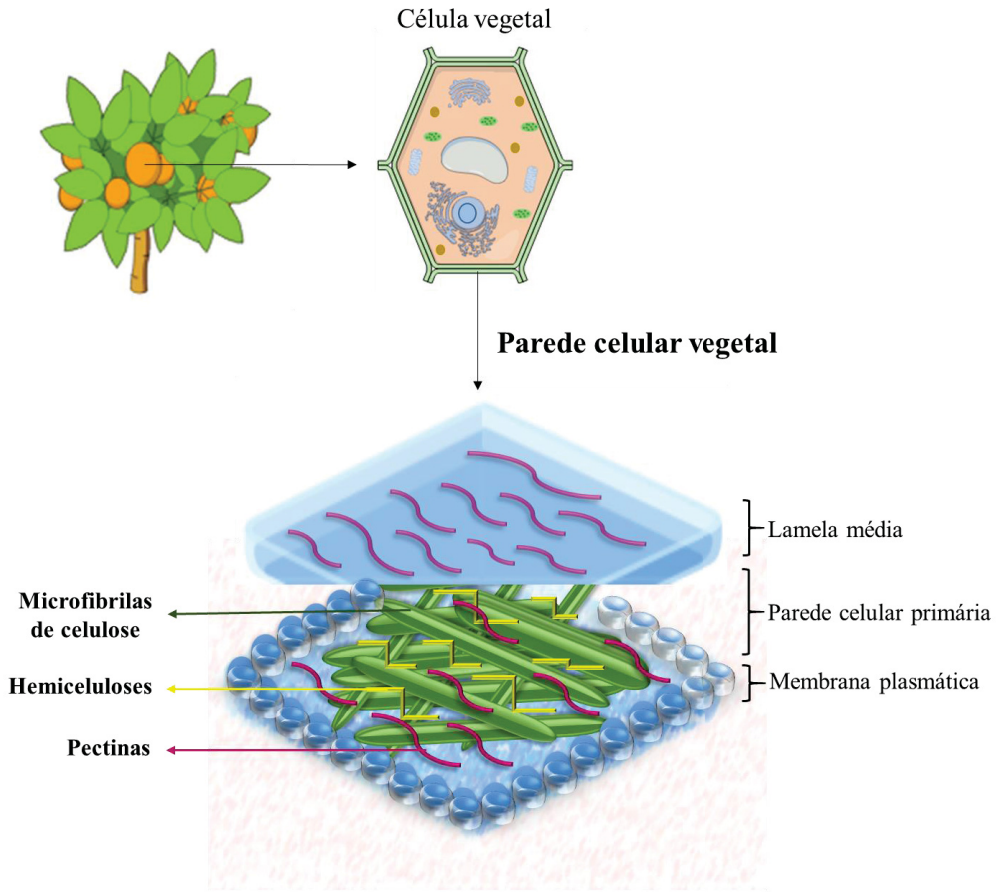
Assim, o presente trabalho esteve inserido desde 2014 no projeto Conservabio II e recentemente, em 2017, no projeto Gabirofood (Gabiroba - caracterização, propagação e tecnologias pós-colheita: potencial de renda para comunidades tradicionais) coordenado pela Dra. Rossana Catie Bueno de Godoy (Embrapa Florestas/Colombo-PR) com o intuito de contribuir para o conhecimento desta espécie. Através da análise estrutural dos polissacarídeos presentes na polpa de gabiroba, do comportamento reológico da polpa *in natura* e de uma formulação de geleia produzida a partir da polpa, foi possível obter informações que agregam valor e dão suporte ao uso sustentável da polpa de gabiroba, a qual pode ser uma fonte de renda alternativa para comunidades rurais onde essa planta é nativa. Além disso, o conhecimento mais específico da composição da fruta, aliado ao seu potencial de aplicação, contribui para estimular seu cultivo auxiliando na preservação desta espécie no Brasil.

2.2 PAREDE CELULAR VEGETAL

A parede celular, localizada externamente à membrana plasmática na célula vegetal, atua na manutenção da estrutura e forma celular, evitando a ruptura da membrana, controlando a entrada de água e outras substâncias na célula, protegendo contra a entrada de patógenos, além de estar envolvida no crescimento celular (McNEIL et al., 1984; CARPITA; GIBEAUT, 1993; CAFFALL; MOHNEN, 2009; BURTON; GIDLEY; FINCHER, 2010).

A parede celular vegetal é uma estrutura altamente organizada composta por polissacarídeos, que correspondem em aproximadamente 90% da sua estrutura, compostos fenólicos e proteínas (CAFFALL; MOHNEN, 2009; BURTON; GIDLEY; FINCHER, 2010). Dentre os polissacarídeos que compõe a parede celular estão a celulose, as hemiceluloses e as pectinas (FIGURA 2) (McNEIL et al., 1984; CARPITA; GIBEAUT, 1993; CAFFALL; MOHNEN, 2009; BURTON; GIDLEY; FINCHER, 2010; HOFTE; VOXEUR, 2017).

FIGURA 2 – REPRESENTAÇÃO ESQUEMÁTICA DA ESTRUTURA DA PAREDE CELULAR VEGETAL



FONTE: O autor (2018).

Durante a divisão celular é formada a lamela média, na qual é encontrada uma grande concentração de pectinas que auxiliam na adesão celular, e também a parede celular primária, que durante o crescimento celular é reorganizada e modificada permitindo a expansão da célula (COSGROVE, 2005). A parede celular primária é constituída por microfibrilas de celulose (25-40%), que possuem alta resistência mecânica, nas quais através de ligações de hidrogênio se ligam as hemiceluloses (15-25%), em meio a uma matriz do tipo gel formada principalmente por polissacarídeos pécticos, que representam cerca de 15 a 40% da parede celular primária (CARPITA; GIBEAUT, 1993; DEY; BROWNLEADER; HARBORNE, 1997; CAFFALL; MOHNEN, 2009; BURTON; GIDLEY; FINCHER, 2010; HOFTE; VOXEUR, 2017). Esta matriz fornece flexibilidade para a célula, e sua porosidade permite que a água e outras moléculas pequenas sejam difundidas através da parede controlando o pH e o balanço iônico (BURTON; GIDLEY; FINCHER, 2010).

A parede celular primária pode ser dividida em tipo I e tipo II, baseado na sua composição (CARPITA; GIBEAUT, 1993). A parede celular primária do tipo I é encontrada

em gimnospermas, monocotiledôneas não-comelinídeas e em dicotiledôneas (classe que inclui a gabiroba - família Myrtaceae). A parede celular do tipo I apresenta maiores quantidades de pectinas e proteínas em relação a parede celular primária do tipo II. Além disso, a xiloglucana destaca-se como principal hemicelulose, podendo ser encontradas também menores quantidades de glucomananas, galactoglucomananas e glucuronoxilanas na parede celular do tipo I (SILVEIRA et al., 2013; BARNES; ANDERSON, 2018). Já a parede celular primária do tipo II, encontrada somente em monocotiledôneas comelinídeas (classe das gramíneas - família Poaceae), apresenta menores quantidades de pectinas e proteínas estruturais, sendo a glucuronoarabinoxilana a principal hemicelulose presente neste tipo de parede (CARPITA; GIBEAUT, 1993; VOGEL, 2008; BARNES; ANDERSON, 2018).

A parede celular secundária é formada após cessar o crescimento celular e pode tornar-se bastante espessa devido à deposição de celulose, hemicelulose e lignina, conferindo maior resistência e rigidez à parede. Em dicotiledôneas em geral, a parede celular secundária é formada por 40-45% de celulose, 15-35% de hemicelulose, 15-30% de lignina e traços de pectina (CARPITA; GIBEAUT, 1993; DEY; BROWNLEADER; HARBORNE, 1997).

Nos frutos, a estrutura da parede celular é modificada durante o processo de amadurecimento. Nesta fase do desenvolvimento, uma série de hidrolases são responsáveis pela degradação das pectinas (principalmente das cadeias laterais, formadas na sua maior parte por unidades de arabinose e galactose) e das xiloglucanas presentes na parede celular. Dessa forma, ocorre um aumento na porosidade da parede facilitando a mobilidade das hidrolases e consequentemente a parede celular torna-se menos rígida (WAKABAYASHI, 2000). Em geral, essas modificações durante a maturação, já descritas na literatura e recentemente observadas por Brahem et al. (2017) para a pera (*Pyrus communis* L.) e por Paniagua et al. (2017) para o morango (*Fragaria x anannasa* Duch.), alteram a textura e a firmeza dos frutos. Estas características são consideradas como principais atributos que garantem a qualidade e a aceitabilidade de frutos *in natura* e de seus produtos industrializados.

2.2.1 Pectinas e suas aplicações

As pectinas são heteropolissacarídeos complexos, que devido a sua vasta gama de aplicações tem despertado cada vez mais o interesse industrial. Em 2013, esses polissacarídeos movimentaram cerca de 850 milhões de dólares no mercado global de hidrocolóides (BOMGARDNER, 2013), e, em 2015, o quilo da pectina foi vendido em média

a 15 dólares, movimentando cerca de um bilhão de dólares na economia a nível mundial (CIRIMINNA, 2016).

No Brasil, em 2017, foram exportadas cerca de 6 mil toneladas de pectinas no valor de 76 milhões de dólares e importadas cerca de 482 toneladas no valor de 6 milhões de dólares, segundo o ministério de desenvolvimento, indústria e comércio exterior (ALICEWEB, 2018).

A produção industrial de pectinas movimenta o mercado global e traz ainda um importante aliado ao mercado de agronegócio ao favorecer o ecossistema por apresentar-se como uma alternativa de agregação de valores aos resíduos sólidos vegetais, sendo que a produção de pectinas geralmente está associada às indústrias de suco, onde são extraídas principalmente a partir da casca de frutas cítricas como limão (56%), lima (30%), laranja (13%), e do bagaço de maçã (14%) (CIRIMINNA, 2016). Uma pequena parte também é obtida dos resíduos da polpa de beterraba, utilizada na produção de açúcar (CANTERI et al., 2012; CIRIMINNA, 2016).

Em geral, as cascas de frutas cítricas apresentam cerca de 25% do seu peso seco em pectinas, as quais na sua maior parte, são passíveis de extração aquosa. Entretanto, extrações sequenciais com oxalato ou ácido etilenodiamino tetra-acético (EDTA) e ácidas com ácido nítrico, ácido cítrico, entre outros, também tem sido empregadas para isolar estes polissacarídeos da parede celular de frutos em escala laboratorial. Para obtenção de pectinas a partir de resíduos industriais de sucos de frutas, a extração em meio ácido sob aquecimento é o método mais utilizado. As condições são variáveis, mas em geral são empregados: pH na faixa de 1,5 a 3,0, em temperaturas de 60 a 100 °C e tempo de 0,5 a 6,0 horas (CANTERI et al., 2012).

As pectinas são polímeros naturais que apresentam uma vasta gama de aplicações devido as suas propriedades funcionais, principalmente como fibras dietéticas (CHENG et al., 2013; ZHANG; XU; ZHANG, 2015; NAQASH et al., 2017). Além disso, as pectinas são biodegradáveis e biocompatíveis, podendo ser aplicadas em diferentes áreas como alimentícia, biomédica, farmacêutica e cosmética (VORAGEN et al., 2009; MUNARIN; PETRINI, 2012; VERONOVSKI et al., 2014; MUNARIN et al., 2015; CHAN et al., 2017; NAQASH et al., 2017; NOREEN et al., 2017).

Para a indústria alimentícia, as pectinas constituem um importante grupo de carboidratos onde são utilizadas como agentes geleificantes, espessantes, texturizantes, emulsificantes e estabilizantes (DICKINSON, 2003; STEPHEN; PHILLIPS; WILLIAMS, 2006; VORAGEN et al., 2009; WICKER et al., 2014; YANG; MU; MA et al., 2018).

Recentemente, esses polissacarídeos vêm sendo propostos na fabricação de filmes comestíveis, associados com outros polissacarídeos como alginato e proteínas, sendo adequados para a incorporação de aditivos como agentes plastificantes, antioxidantes, antimicrobianos e óleos essenciais (SILVA; BIERHALZ; KIECKBUSCH, 2009; NISAR et al., 2018). Os filmes comestíveis apresentam-se como uma nova alternativa às embalagens sintéticas para a proteção e conservação de alimentos. Além disso, Krivorotova et al. (2016) relataram o uso das pectinas como revestimento em sistemas de nanopartículas com agentes antimicrobianos, por exemplo nisina, para a preservação de alimentos.

As pectinas também apresentam potencial para produção de biomateriais, como suportes (*scaffold*), hidrogéis e nanopartículas, que podem ser aplicados na engenharia de tecidos (COIMBRA et al., 2011; MUNARIN et al., 2015) e na liberação controlada de fármacos (MUNARIN; TANZI; PETRINI, 2012; JUNG; ARNOLD; WICKER, 2013; PRAMANIK; GANGULY, 2017). Além disso, Pallavicini et al. (2017) também mostraram o potencial de pectinas como revestimento de nanopartículas de prata onde promovem a proliferação de fibroblastos, auxiliando nos processos de cicatrização de feridas e atuando como barreira contra infecção bacteriana em procedimentos pós cirúrgicos.

Em relação as propriedades biológicas, as pectinas vêm se destacando pelas suas propriedades antioxidantes (DALONSO; PETKOWICZ, 2012; KLOSTERHOFF et al., 2018), anti-inflamatórias (TAMIELLO et al., 2018) e antitumorais (ZHANG; XU; ZHANG, 2015). Quanto as propriedades antiumorais, os estudos têm focado principalmente no câncer de cólon relacionando essas propriedades com a atividade prébiótica das pectinas e sua função como fibra solúvel (CHENG et al., 2013; ZHANG; XU; ZHANG, 2015; NAQASH et al., 2017). Homogalacturonanas e ramnogalacturonanas também estão sendo investigadas quanto ao seu papel na inibição da migração celular através da diminuição da adesão celular, sendo a migração celular relacionada ao desenvolvimento de patologias como inflamações crônicas e progressão de células cancerígenas (FAN et al., 2018).

As pectinas são os polissacarídeos mais complexos da parede celular das plantas, constituindo até 40% da parede celular de frutas e vegetais (CARPITA; McCANN, 2000; CAFFALL; MOHNEN, 2009). Apresentam um importante papel na adesão célula-célula e contribuem para a retenção de água e formação de géis, influenciando as propriedades mecânicas da parede celular, tais como porosidade, fluxo de íons e manutenção do pH (CARPITA; McCANN, 2000; MOHNEN, 2008; VORAGEN et al., 2009). As pectinas também podem ativar o sistema de defesa das plantas estimulando o acúmulo de enzimas e fitoalexinas, as quais apresentam atividade antimicrobiana e atuam na proteção da parede

celular contra a infecção por bactérias ou fungos (CAFFALL; MOHNEN, 2009; BENEDETTI et al., 2015).

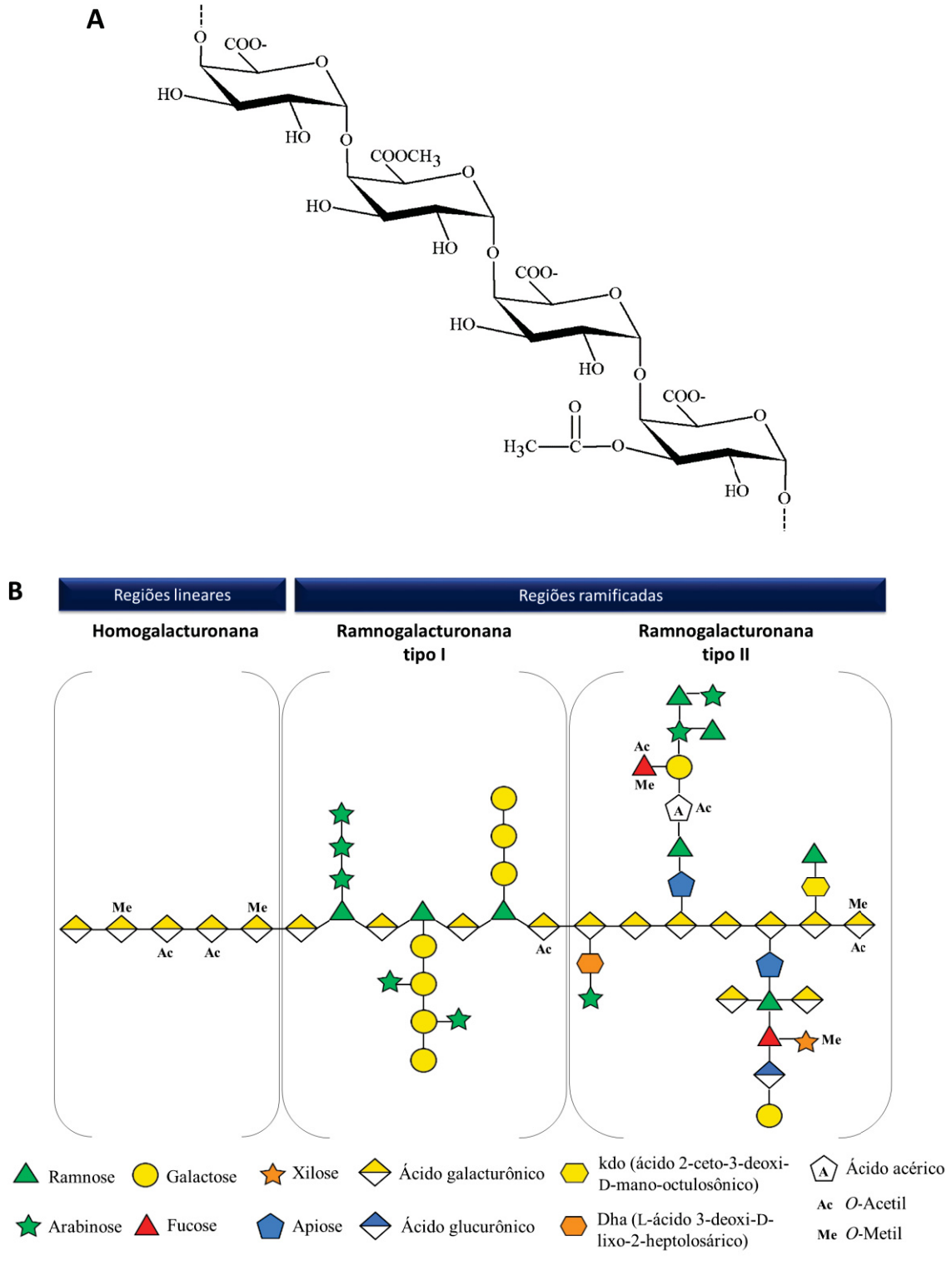
As pectinas podem ser compostas por até 17 monossacarídeos diferentes, dentre os quais o ácido D-galacturônico (D-GalA) é o mais abundante, seguido por D-galactose (D-Gal) e L-arabinose (L-Ara) (YAPO, 2011a). As estruturas variam entre as espécies, tecidos e até mesmo entre as diferentes partes de um fruto. Os principais domínios que compõem as pectinas são: homogalacturonanas (HG), ramnogalacturonanas do tipo I (RG-I) e ramnogalacturonanas do tipo II (RG-II) (FIGURA 3) (CARPITA; GIBEAUT, 1993; WILLATS et al., 2006; CAFFAL; MOHNEN 2009).

As homogalacturonanas (HG) (FIGURA 3) representam cerca de 65% da estrutura das pectinas presentes na parede celular, sendo formadas por unidades de α-D-GalA*p* (1→4) ligadas, constituindo a região linear ou *smooth region* (CARPITA; GIBEAUT, 1993; MOHNEN, 2008; YAPO, 2011a). As unidades de α-D-GalA*p* da cadeia linear das HG podem estar parcialmente metil-esterificadas em C-6 e acetil-esterificadas nas posições *O*-2 e/ou *O*-3 (FIGURA 3A) (MOHNEN, 2008; YAPO, 2011a), podendo o grau de metil-esterificação e acetilação influenciar no comportamento de geleificação e nas propriedades funcionais das pectinas (WILLATS et al., 2006; CHAN et al., 2017).

Dependendo do grau de metil-esterificação (DM ou DE), as pectinas podem ser divididas em: **1-** pectinas com alto teor de metil-esterificação (HM - *High methoxyl pectin*), as quais apresentam DE superior a 50%, e são caracterizadas por formar géis em pH ácido inferior a 3,5, na presença de altas concentrações (60-65%) de co-solutos como a sacarose; **2-** pectinas com baixo teor de metil-esterificação (LM – *Low methoxyl pectin*), que possuem DE inferior a 50%, apresentam-se mais solúveis e são capazes de formar gel na presença de íons cálcio (Ca^{2+}) (VORAGEN et al., 1995; BEMILLER, 1996; STEPHEN; PHILLIPS; WILLIAMS, 2006; CHAN et al., 2017).

As ramnogalacturonanas do tipo I (RG-I) (FIGURA 3B) representam cerca de 20-35% das pectinas e são formadas por uma cadeia principal de unidades alternadas de α-D-GalA*p* (1→4) ligadas, e unidades de α-L-Rha*p* (1→2) ligadas, as quais podem estar parcialmente substituídas nas posições *O*-4 e/ou *O*-3 (menos frequente), por cadeias laterais formadas por α-L-arabinanas (1→5) ligadas, β-D-galactanas (1→4) ligadas, arabinogalactanas do tipo I (AG-I) e arabinogalactanas do tipo II (AG-II) (VORAGEN et al., 2009; YAPO, 2011a). Essas estruturas constituem as regiões denomidadas ramificadas ou *hairy regions* das cadeias de pectinas (YAPO, 2011a).

FIGURA 3 – REPRESENTAÇÃO ESQUEMÁTICA DA ESTRUTURA DAS PECTINAS. (A) HOMOGALACTURONANA COM CADEIA PRINCIPAL FORMADA POR UNIDADES DE α-D-GalA*p* (1→4) LIGADAS. (B) ESQUEMA DAS ESTRUTURAS DE HOMOGALACTURONANAS, RAMNOGALACTURONANAS DO TIPO I E RAMNOGALACTURONANAS DO TIPO II



FONTE: O autor (2018), de acordo com a nomenclatura de símbolos para glicanos (VARKI et al., 2015).

As ramnogalacturonanas do tipo II (RG-II) (FIGURA 3B) são encontradas em menores quantidades (aproximadamente 10% das pectinas) formando estruturas complexas. A cadeia principal das RG-II são formadas por 8 a 10 unidades de α-D-GalA*p* (1→4) ligadas, substituídas em *O*-2 e/ou *O*-3 por cadeias laterais heteropoliméricas. As cadeias laterais das RG-II podem conter monossacarídeos raros como apiose (3-*C*-hidroximetil-β-D-eritriose), ácido acérico (3-*C*-carboxi-5-deoxi-L-xilose), kdo (ácido 2-ceto-3-deoxi-D-mano-octulosônico), dha (L-ácido 3-deoxi-D-lixo-2-heptolosárico), 2-*O*-metil-L-Fuc*p* e 2-*O*-metil-D-Xyl*p*, unidos por mais de 20 ligações distintas (YAPO, 2011b).

Considerando o alto valor agregado e o potencial de aplicação em diferentes áreas, as pectinas provenientes de frutas vêm sendo cada vez mais estudadas no mundo todo. Dentre os centros de investigação na área de carboidratos, pode-se destacar o Grupo de Química de Carboidratos do Departamento de Bioquímica e Biologia Molecular da Universidade Federal do Paraná, ao qual este estudo está inserido, que vem estudando há 30 anos a composição química de pectinas extraídas de diferentes espécies de frutas. Na tabela 1 são apresentados alguns estudos mais recentes com enfoque na estrutura química destes polissacarídeos, sendo ressaltados com asterisco (*) os trabalhos desenvolvidos pelo Departamento citado acima.

TABELA 1 – ESTUDOS DA EXTRAÇÃO E CARACTERIZAÇÃO QUÍMICA DAS PECTINAS OBTIDAS DE DIFERENTES ESPÉCIES DE FRUTAS

(continua)

Espécie	Parte da fruta estudada	Extração	Estrutura química dos polissacarídeos	Referências
Melancia (*Citrullus lanatus*)	Casca	Extração ácida sob refluxo Ácido nítrico (0,1M, 1h)	HG e RG-I Galactanas	* [1]
Cubiu (*Solanum sessiflorum* D.)	Casca	Extração sequencial Água (100 ºC, 2h) EDTA (0,05 M, 4h) Ácido cítrico (pH 2.5, 70 ºC, 30 min)	HG RG-I AG-I AG-II	* [2]
Cacau (*Theobroma cacao* L.)	Casca	Extração ácida Ácido nítrico (pH 3,5, 100 ºC, 30 min)	Pectinas altamente acetiladas RG-I Galactanas	* [3]
Pimentão (*Capsicum annum*)	Fruta com pele, sem sementes	Extração aquosa sob refluxo (100 ºC, 2h)	HG RG-I AG-I e AG-II	* [4]
Acerola (*Malpiglia emarginata*)	Polpa sem casca e sem sementes	Extração aquosa sob refluxo (100 ºC, 2h, 6x)	Arabinana	* [5]
Pupunha (*Bactris gasipaes*)	Polpa	Extração aquosa (100 ºC, 2h)	HG RG-I Xilogalacturonana	* [6]
Carambola (*Averrhoa carambola* L.)	Fruta sem semente	Extração aquosa sob refluxo (100 ºC, 2h, 6x)	AG-II	* [7]

(conclusão)

Espécie	Parte da fruta estudada	Extração	Estrutura química dos polissacarídeos	Referências
Cupuaçu (*Theobroma grandiflorum*)	Polpa	Extração aquosa (25 ºC, 90 min, 2x) (60 ºC, 180 min)	HG RG-I	* [8]
Pitaia (*Hylocereus* spp.)	Casca	Extração ácida Ácido cítrico em diferentes concentrações (50 ºC, 60 min) Extração assistida por micro-ondas (*MAE*) (ácido nítrico, pH 2,0) (70-100 ºC) (300-600 W, 5-10 min)	HG com alto grau de metil-esterificação	[9]
Romã (*Punica granatum*)	Casca	Extração aquosa ácida Ácido nítrico 20 mM (86 ºC, 80 min)	HG	[10]
Maçã (*Malus domestica*)	Bagaço	Extração aquosa subcrítica em autoclave (130-170 ºC, 5 min)	RG-I Arabinana Arabinogalactana	[11]
Maracujá (*Passiflora edulis*)	Casca	Extração por micro-ondas Ácidos tartárico, acético e nítrico (pH 2,0) (3, 6 e 9 min)	HG RG-I	[12]
Goji berry (*Lycium ruthenicum*)	Fruta inteira	Extração aquosa (70 ºC, 2h)	HG RG-I	[13]

NOTA: (*) Trabalhos desenvolvidos pelo Grupo de Química de Carboidratos do Departamento de Bioquímica e Biologia Molecular da Universidade Federal do Paraná. (HG) Homogalacturonanas. (RG-I) Ramnogalacturonanas do tipo I. (AG-I) Arabinogalactanas do tipo I. (AG-II) Arabinogalactanas do tipo II. [**1**] PETKOWICZ; VRIESMANN; WILLIAMS, 2017; [**2**] COLODEL et al., 2017; [**3**] VRIESMANN; PETKOWICZ, 2017; [**4**] NASCIMENTO; IACOMINI; CORDEIRO, 2017; [**5**] KLOSTERHOFF et al., 2018; [**6**] CANTU-JUNGLES et al., 2017; [**7**] LEIVAS; IACOMINI; CORDEIRO, 2016; [**8**] VRIESMANN; PETKOWICZ, 2009; [**9**] TONGKHAM et al., 2017; [**10**] ABID et al., 2017; [**11**] WANG; CHEN; LU et al., 2014; [**12**] SEIXAS et al., 2014; [**13**] PENG et al., 2014. FONTE: O autor (2018).

Apesar da existência de diversos estudos descrevendo a caracterização da estrutura química de pectinas, até o momento são escassos os trabalhos relacionados as frutas pertencentes a família Myrtaceae. Em geral, a literatura disponível para estas frutas, apresenta somente dados sobre a composição química e nutricional, como quantidade de proteínas, lipídeos, carboidratos totais, fibras, vitaminas e compostos fenólicos (ALEZANDRO et al., 2013; CHAVES et al., 2013).

Na tabela 2 são apresentados os estudos encontrados até o momento em relação à caracterização da estrutura química, físico-química e da atividade biológica dos polissacarídeos pécticos extraídos das espécies frutíferas da família Myrtaceae, dentre as quais a goiaba (*Psidium guajava*) destaca-se como a espécie mais estudada.

Em relação à gabiroba, os dados presentes na literatura mostram apenas a composição monossacarídica de uma fração péctica obtida por extração aquosa a temperatura ambiente, que apresentou Ara (74,9%), Gal (16,8%), Glc (3,6%), Xyl (2,9%), Man (0,8%), Rha (0,8%) e ácido urônico (16,9%), em sua composição (SANTOS et al., 2012).

Dessa forma, buscando-se aprofundar os conhecimentos em relação à estrutura química das pectinas presentes na polpa de gabiroba, no Artigo I do presente estudo, são apresentados os resultados em relação a obtenção de polissacarídeos pécticos extraídos da polpa de gabiroba, bem como o processo de purificação e caracterização da estrutura química das frações obtidas, além das análises do comportamento reológico da fração de maior rendimento.

TABELA 2 – ESTUDOS DE EXTRAÇÃO E CARACTERIZAÇÃO QUÍMICA DAS PECTINAS OBTIDAS DAS ESPÉCIES DE FRUTAS DA FAMÍLIA MYRTACEAE

(Continua)

Espécie	Parte da fruta estudada	Extração	Estrutura química dos polissacarídeos	Observações	Referências
Gabiroba (*Campomanesia xanthocarpa* Berg)	Polpa da fruta sem sementes	Extração aquosa (26 ºC, 60 min)	Composição monossacarídica - presença de pectinas	Análise do comportamento reológico das pectinas	[1]
Jambo (*Syzygium jambo*)	Fruta inteira com a casca	Extração aquosa em banho fervente sob refluxo (2h, 7x)	HG RG-I AG-II	Atividade antiinflamatória relacionada a fração de AG-II	[2]
Murta comum (*Myrtus communis* L.)	Polpa e casca, sem sementes	Extração aquosa (80 ºC, 24 h)	RG-I AG-I Xiloglucanas		[3]
Murta (*Ugni molinae* Turcz)	Fruta inteira	Extração sequencial: Água (20 ºC, 30 min, 3x) Oxalato de amônio (20 ºC, 30 min, 3x) HCl (85 ºC, 30 min, 3x)	Fração aquosa: RG-I Fração oxalato e fração ácida: HG		[4]
Cambuí (*Myrciaria tenella* Berg)	Fruta inteira	Extração sequencial: Água (25 ºC, 4h) EDTA 2% (25 ºC, 6h) Na_2CO_3 (25 ºC, 6 h)	RG-I Arabinanas Galactanas		[5]
Feijoa (*Acca sellowiane*)	Polpa	Extração acelerada por solvente (*ASE*) Extração água: ácido cítrico (20 ºC, pH 3,5, 1500 psi, 3x)	Composição monossacarídica – presença de pectinas	Atividade antioxidante	[6]

(conclusão)

Espécie	Parte da fruta estudada	Extração	Estrutura química dos polissacarídeos	Observações	Referências
Jaboticaba sabará (*Myrciaria cauliflora*)	Casca Polpa Suco da polpa	Extração aquosa (80 ºC, 10 min), seguido de adição de ácido nítrico 0,1 M mantendo a extração por 20 min, 97ºC, sob refluxo	HG RG-I		[7]
	Fruta (mesocarpo e endocarpo sem sementes)	Extração aquosa	RG-I AG-I AG-II		[8]
Goiaba (*Psidium guajava*)	Fruta inteira	Extração aquosa (80 ºC, 3h, 3x)		Atividade antioxidante	[9]
	Fruta inteira	Extração aquosa (80 ºC, 3h)	Cadeia principal de arabinana α-L-Ara (1→5) ligada α-L-Ara (1→3) ligada Cadeia lateral com unidades de glucose e arabinose	Atividade antioxidante	[10]
Araçá (*Psidium cattleianum*)	Polpa	Extração aquosa (25 ºC, 3h) EDTA (25 ºC, 6h	Composição monossacarídica		[11]

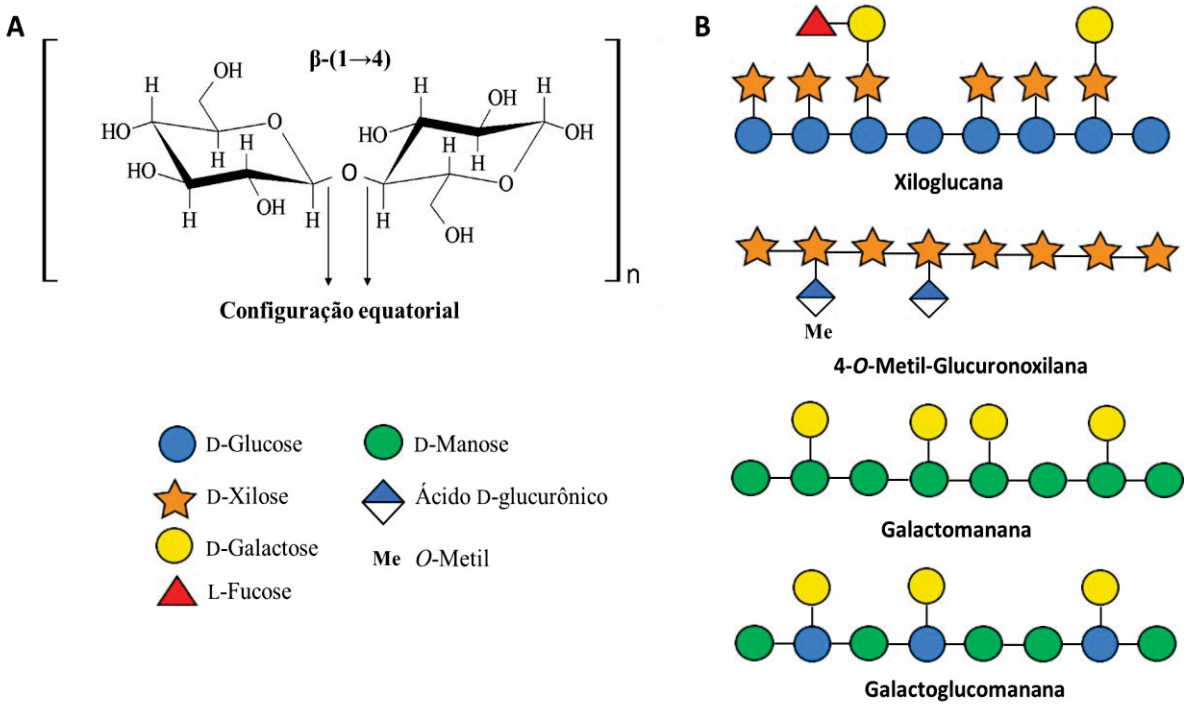
NOTA: (HG) Homogalacturonanas. (RG-I) Ramnogalacturonanas do tipo I. (AG-I) Arabinogalactanas do tipo I. (AG-II) Arabinogalactanas do tipo II. [**1**] SANTOS et al., 2012; [**2**] TAMIELLO et al., 2018; [**3**] CHIOUH; AOUADI; HEYRAUD, 2014; [**4**] TABOADA et al., 2010; [**5**] VRIESMANN et al., 2004; [**6**] SUN-WATERHOUSE et al., 2012; [**7**] MORENO et al., 2016; [**8**] MARCELIN; SAUNIER; BRILLOUET, 1991; [**9**] HUA et al., 2014; [**10**] ZHANG et al., 2016. [**11**] VRIESMANN et al., 2009.
FONTE: O autor (2018).

2.2.2 Hemiceluloses e suas aplicações

As hemiceluloses são encontradas na parede celular vegetal, onde interagem com as microfibrilas de celulose, contribuindo principalmente para o fortalecimento da parede celular (CARPITA; GIBEAUT, 1993; CAFFALL; MOHNEN, 2009; BURTON; GIDLEY; FINCHER, 2010; SILVEIRA et al., 2013). As hemiceluloses em geral, são descritas como polímeros que podem ser extraídos da parede celular com solventes alcalinos, e apresentam uma cadeia principal formada por unidades monossacarídicas na forma anomérica β e (1→4) ligadas em posição equatorial no C-1 e C-4 (FIGURA 4A) (CAFFALL; MOHNEN, 2009; BURTON; GIDLEY; FINCHER, 2010; SCHELLER; ULVSKOV, 2010).

As hemiceluloses são um grupo heterogêneo de polissacarídeos que variam sua composição e estrutura de acordo com a espécie de planta em que são encontradas. Os principais tipos são as xilanas, 4-*O*-metil-glucuronoxilanas, xiloglucanas, mananas, glucomananas e galactoglucomananas (FIGURA 4B) (SCHELLER; ULVSKOV, 2010).

FIGURA 4 - HEMICELULOSES. (A) REPRESENTAÇÃO DA LIGAÇÃO (1→4) EM CONFIGURAÇÃO EQUATORIAL NO C-1 E C-4 EM HEMICELULOSES. (B) MODELO ESQUEMÁTICO DE ESTRUTURAS DE HEMICELULOSES ENCONTRADAS EM PLANTAS



FONTE: O autor (2018), de acordo com a nomenclatura de símbolos para glicanos (VARKI et al., 2015).

As xilanas apresentam cadeias lineares formadas por unidades de β-D-xilopiranose (1→4) ligadas, as quais podem estar substituídas na posição C-2 e/ou C-3 por cadeias laterais curtas e flexíveis formando as heteroxilanas (EBRINGEROVÁ et al., 2005; BURTON; GIDLEY; FINCHER, 2010). Nas plantas dicotiledôneas, os principais substituintes são as unidades de ácido α-D-glucurônico ou ácido 4-*O*-metil-α-D-glucurônico (na parede celular sencundária), sendo as estruturas chamadas glucuronoxilanas ou 4-*O*-metil-glucuronoxilanas. Unidades de α-L-arabinofuranose, α-D-xilopiranose ou α-D-galactopiranose também podem ser encontradas como substituintes da cadeia principal das xilanas, formando as arabinoxilanas e as glucuronoarabinoxilanas (EBRINGEROVÁ et al., 2005; BURTON; GIDLEY; FINCHER, 2010; SCHELLER; ULVSKOV, 2010; NAIDU; HLANGOTHI; JOHN, 2018).

As xiloglucanas são as hemiceluloses mais abundantes encontradas nas dicotiledôneas compondo cerca de 20 a 25% da parede celular primária (CAFFALL; MOHNEN, 2009; SCHELLER; ULVSKOV, 2010). Estes polissacarídeos apresentam uma cadeia principal formada por unidades de β-D-glucopiranose (1→4) ligadas, sendo que até 70% destas unidades podem ser substituídas na posição C-6 por unidades de α-D-xilopiranose. Unidades de β-D-galactose, α-L-arabinose e α-L-fucose também podem ser encontradas ligadas as unidades de xilose na cadeia lateral das xiloglucanas (BUSATO et al., 2005; EBRINGEROVÁ et al., 2005; BURTON; GIDLEY; FINCHER, 2010; SCHELLER; ULVSKOV, 2010; LUCYSZYN et al., 2011; NAIDU; HLANGOTHI; JOHN, 2018).

As mananas podem ser divididas em mananas lineares, galactomananas, glucomananas e galactoglucomananas (YILDIZ; ONER, 2014). As mananas lineares são estruturas insolúveis em água, compostas por cadeia principal de unidades de β-D-manopiranose (1→4) ligadas (PETKOWICZ et al., 2001; EBRINGEROVÁ et al., 2005, BENTO et al., 2013). As galactomananas, encontradas principalmente no endosperma das sementes de leguminosas, apresentam cadeia principal formada por unidades de β-D-manopiranose (1→4) ligadas, substituídas na posição *O*-6 por unidades de α-D-galactopiranose (GANTER et al., 1992; GANTER et al., 1993; PETKOWICZ et al., 1998; SOUZA et al., 2010; SALVALAGGIO et al., 2015; YILDIZ; ONER, 2014). As unidades de α-D-galactopiranose da cadeia lateral influenciam na solubilidade das galactomananas, sendo necessário que no mínimo 10% da cadeia seja substituída para que a estrutura seja solúvel em água (DEA; MORRISON, 1975; SILVEIRA; BRESOLIN, 2011).

As glucomananas são formadas por uma cadeia principal composta por unidades de β-D-manopiranose e β-D-glucopiranose (1→4) ligadas, sendo a proporção entre estes

monossacarídeos variável de acordo com a fonte de onde são extraídos (EBRINGEROVÁ et al., 2005; ZIA et al., 2016; NAIDU; HLANGOTHI; JOHN, 2018). As glucomananas podem ser parcialmente acetiladas, com o grau de acetilação variando de 5 a 10%, o que pode conferir caráter geleificante a estes polissacarídeos (CAMPESTRINI et al., 2013; ZIA et al., 2016).

As galactoglucomananas apresentam cadeia principal composta por unidades de β-D-manopiranose e β-D-glucopiranose (1→4) ligadas, assim como as glucomananas, no entanto, nas galactoglucomananas são encontradas unidades de α-D-galactopiranose ligadas na posição *O*-6 das unidades de glucose ou de manose (NARA et al., 2004; BARBIERI et al., 2017; NAIDU; HLANGOTHI; JOHN, 2018). As unidades de manose da cadeia principal das galactoglucomananas também podem ser parcialmente acetiladas no C-2 ou C-3, sendo essas estruturas as principais hemiceluloses encontradas na parede celular secundária das coníferas (*softwoods*) (CAPEK et al., 2000; XU et al., 2010).

As galactoglucomananas foram relatadas na parede celular primária de frutos como o kiwi (*Actinidia deliciosa*) (SCHRÖDER et al., 2001) e a maçã (*Malus domestica*) (NARA et al., 2004), e recentemente também foram extraídas e purificadas da polpa de gabiroba, cujos resultados são apresentados e discutidos no artigo II do presente estudo (BARBIERI et al., 2017).

O conhecimento sobre a ocorrência, purificação e estrutura química das hemiceluloses abre novas perspectivas para a exploração da fonte de onde são obtidos, além de auxiliar no direcionamento desses polissacarídeos em diferentes aplicações biológicas e biotecnológicas.

As hemiceluloses em geral, apresentam potencial para uma variedade de aplicações podendo ser utilizadas como aditivos na indústria alimentícia (PRAJAPATI et al., 2013; YILDIZ; ONER, 2014), base para formulação de biofilmes e hidrogéis (HARTMAN; ALBERTSSON; SJÖBERG, 2006; MIKKONEN et al., 2010; MARKSTEDT et al., 2017; MENDES et al., 2017; NAIDU; HLANGOTHI; JOHN, 2018), sistemas nanocarreadores de fármacos e agentes terapêuticos (EBRINGEROVÁ et al., 2008; WILLFÖR et al., 2008; SILVEIRA; BRESOLIN, 2011; YILDIZ; ONER, 2014). Além disso, esses polissacarídeos podem ser incorporados na base de materiais sintéticos, como poliuretanos, melhorando a biodegradabilidade e biocompatibilidade destes materiais (ZIA et al., 2016).

2.3 REOLOGIA

A reologia é a ciência que estuda a deformação e o fluxo dos materiais sob a influência de uma determinada tensão (BARNES; HUTTON; WALTERS, 1989; SCHRAMM, 2006). Os materiais em geral, podem ser definidos como sólidos ou líquidos. Os sólidos ideais se deformam elasticamente, sendo a energia necessária para esta deformação recuperada completamente quando a tensão é removida. Já os líquidos considerados fluidos ideais, quando submetidos a uma tensão, deformam-se irreversivelmente de forma que a energia empregada para que este material flua é dissipada na forma de calor, não sendo recuperada após a retirada da tensão (TONELI; MURR; PARK, 2005; SCHRAMM, 2006).

Materiais intermediários entre os sólidos e fluidos ideais são denominados viscoelásticos, e podem apresentar comportamento reológico mais próximo ao elástico tal como os sólidos, ou mais viscoso, tal como os líquidos, dependendo da tensão, frequência ou temperatura a que são submetidos (BARNES; HUTTON; WALTERS, 1989; SCHRAMM, 2006).

O comportamento reológico dos fluidos pode ser caracterizado através da análise de curva de fluxo em regime estacionário, por meio da relação entre a tensão de cisalhamento e a taxa de cisalhamento. Além disso, através de modelos matemáticos empíricos como Ostwald de Waelle, Herschel-Bulkley, Power Law**,** Cross, Casson (CASSON, 1959; CROSS, 1965; STEFFE, 1996; RAO, 2007), é possível descrever o comportamento reológico dos fluidos, os quais podem ser divididos em newtonianos e não newtonianos (STEFFE, 1996; TONELI; MURR; PARK, 2005).

Nos fluidos Newtonianos, a viscosidade, definida como a resistência do fluido em relação ao fluxo induzido pelo cisalhamento, é independente da taxa de cisalhamento. Já para os fluidos não Newtonianos (pseudoplásticos, plásticos e dilatantes) a viscosidade varia com a taxa de cisalhamento (SCHRAMM, 2006).

Dentre os fluidos denominados não Newtonianos, destacam-se os fluidos pseudoplásticos, os quais são caracterizados pela diminuição da viscosidade aparente com o aumento da taxa de cisalhamento. Ou seja, nos fluidos pseudoplásticos as partículas e/ou cadeias poliméricas mantém uma ordem interna irregular (desorientada) quando em repouso, gerando uma alta viscosidade. No entanto, com o aumento da taxa de cisalhamento, essas cadeias poliméricas se reorganizam e se alinham em direção ao fluxo escoando de maneira mais fácil e consequentemente diminuindo a viscosidade do fluido (SCHRAMM, 2006; RAO, 2007).

O comportamento reológico de um material também pode ser caracterizado através das análises oscilatórias dinâmicas, a partir das quais se obtem informações sobre a viscosidade e a elasticidade do material quando submetido a tensões oscilatórias. O estudo do comportamento viscoelástico é baseado na dependência dos módulos G′ (módulo de armazenamento ou elástico) e G″ (módulo viscoso ou de perda) em função da frequência (MORRIS, 1995; NISHINARI, 2006; SCHRAMM, 2006). Como resultado dos testes oscilatórios, quando o módulo G′ é maior que o módulo G″ em toda faixa de frequência analisada, o material apresenta caráter elástico ou de gel. Quando os valores de G″ apresentam-se maiores do que G′ em baixas frequências, mas em altas frequências ocorre uma inversão nos módulos, o comportamento é típico de soluções concentradas. Já em soluções diluídas, é observado que o módulo G″ se mantém maior do que G′ em toda faixa de frequência (MORRIS, 1995; NISHINARI, 2006; SCHRAMM, 2006).

2.3.1 Reologia de polissacarídeos

Grande parte das aplicações dos polissacarídeos, em especial das pectinas, envolve sua habilidade em alterar as propriedades físicas do meio onde se encontram, conferindo viscosidade as soluções ou criando redes intermoleculares coesivas formando géis (STEFFE, 1996).

Como mencionado anteriormente, as pectinas podem ser classificadas em HM e LM, com alto ou baixo grau de metil-esterificação, respectivamente, o que influencia no mecanismo de geleificação. Deste modo, trabalhos na literatura, mostram a influência do cálcio (Ca^{2+}) no comportamento reológico das pectinas LM (KYOMUGASHO et al., 2016; ABID et al., 2017), bem como a influência de sacarose na formação de gel nas pectinas HM (EVAGELIOU; RICHARDSON; MORRIS, 2000; VRIESMANN; PETKOWICZ, 2013; GAMONPILAS et al., 2015; NASCIMENTO et al., 2016).

Além disso, as análises reológicas vêm sendo empregadas para analisar o comportamento de misturas de pectinas com outros polissacarídeos e proteínas buscando ampliar seu potencial de aplicação. Neste contexto, Lofgren e Hermansson (2007) observaram que a mistura de pectinas LM e HM podem formar géis mais fortes comparado com géis de pectinas HM com sacarose. Agudelo, Varela e Fiszman (2014) mostraram que a mistura entre pectinas e amido de tapioca aumentou a estabilidade de pures de frutas, reduzindo o processo de sinérese (liberação de água) durante o congelamento. Chang et al. (2017) também

avaliaram o potencial da pectina como substituinte da gordura (óleo) adicionada no preparo de maionese, através da interação pectina-proteina do ovo.

A literatura também vem mostrando que a viscosidade das soluções de pectinas (em água ou na presença de sais) tende a aumentar de acordo com o aumento da concentração deste polissacarídeo. Esse comportamento já foi relatado para pectinas extraídas de manga (*Mangifera indica* L.) (IAGHER; REICHER; GANTER, 2002), toranja (*Citrus paradisi*) (WANG et al., 2016), tamarillo (*Solanum betaceum*) (NASCIMENTO et al., 2016), cubiu (*Solanum sessiliflorum* Dunal) (COLODEL et al., 2017), melancia (*Citrullus lanatus*) (PETKOWICZ; VRIESMANN; WILLIAMS, 2017), entre outros.

Em relação as pectinas extraídas de frutas da família Myrtaceae, até o momento foi encontrado apenas um estudo prévio com frações pécticas extraídas da polpa de gabiroba. Neste estudo foi analisado o comportamento reológico das frações extraídas com água (26 ºC), ácido cítrico (50 ºC e 100 ºC) e NaOH (26 ºC) durante 60 min. Com as frações pécticas foram feitas soluções a 30 g L^{-1} em NaCl 0,1 mol L^{-1}, na presença de cálcio (20%). O comportamento reológico foi avaliado através de curva de fluxo a 20 ºC, na qual foi observado um comportamento pseudoplástico. O comportamento viscoelástico das amostras também foi analisado. A varredura de frequência na faixa de 0,1-10 Hz mostrou que módulo de armazenamento (G') se manteve superior ao módulo de perda (G") em toda a faixa de frequência analisada, evidenciando a presença de uma rede típica de um gel (SANTOS et al., 2012).

No Artigo I do presente estudo, são abordados os resultados referentes ao comportamento reológico da fração péctica de maior rendimento, extraída da polpa de gabiroba através de extração com água fervente sob refluxo.

2.3.2 Reologia de polpa de frutas e geleias

A demanda por produtos nativos e exóticos, particularmente para produtos alimentícios, vem crescendo, criando a necessidade de uma melhor compreensão da influência do processamento na estrutura e propriedades destes produtos (AYALA-ZAVALA et al., 2011).

Considerando a busca por alimentos processados de alta qualidade, o conhecimento reológico das propriedades viscoelásticas da matéria prima, é um fator importante para o projeto, otimização, estabilidade e desenvolvimento de produtos visando uma maior aceitação

pelo consumidor final (FISCHER; WINDHAB, 2011; AUGUSTO; CRISTIANINI; IBARZ, 2012).

As polpas de frutas, devido ao seu alto valor nutricional, vêm sendo cada vez mais utilizadas como matéria prima para a produção de produtos alimentícios e cosméticos. Dessa forma, o conhecimento das propriedades reológicas é de grande interesse e polpas de diferentes frutas têm sido investigadas. Na tabela 3 são apresentados alguns estudos do comportamento reológico de polpas de frutas, tais como: a polpa de manga (*Mangifera indica*) (AHMED; RAMASWAMY; HIREMAYH, 2005), butia (*Butia capitata*) (HAMINIUK et al., 2006a), açaí (*Euterpe oleraceae* Mart.) (TONON et al., 2009), siriguela (*Spondias purpurea* L.) (AUGUSTO et al., 2012), e pequi (*Caryocar coriaceum*) (SOUSA et al., 2014). Em grande parte dessas polpas, foi analisado, em geral através de experimentos de curvas de fluxos, a influência da temperatura no comportamento reológico, sendo observado que a viscosidade das polpas diminuiu com o aumento de temperatura, e que todas apresentaram comportamento pseudoplástico.

Como mencionado anteriormente, as frutas pertencentes a família Myrtaceae ainda são pouco exploradas, sendo suas propriedades reológicas pouco conhecidas. Na tabela 3 são apresentados estudos referentes ao comportamento reológico de polpas de frutas de algumas espécies de Myrtaceae, destacadas com asterisco (*), como a jabuticaba (*Myrciaria cauliflora*) (SATO; CUNHA, 2007), o araçá (*Psidium cattleianum* sabine) (HAMINIUK et al., 2006b), e a goiaba (*Psidium guajava*) (DE OLIVEIRA; ROSSI; DE BARROS, 2011; PEREIRA et al., 2012). Estas frutas tiveram suas polpas caracterizadas através de análises reológicas principalmente em relação ao comportamento de fluxo em diferentes temperaturas. Os dados experimentais obtidos para cada polpa foram ajustados em diferentes modelos matemáticos, e todos apresentaram comportamento pseudoplástico (TABELA 3).

Em relação à gabiroba, até o momento foram encontrados trabalhos, somente caracterizando o comportamento da polpa como pseudoplástico, através dos modelos matemáticos empíricos de Ostwald de Waele e Herschel-Bulkley (TABELA 3) (DE OLIVEIRA; ROSSI; DE BARROS, 2011; SANTOS et al., 2012). Assim, no artigo III do presente estudo, a polpa de gabiroba *in natura*, foi analisada quanto ao seu comportamento reológico, buscando-se caracterizar essa polpa para o uso como matéria prima principalmente para a produção de geleias. Neste estudo a reologia também foi utilizada para analisar a estabilidade térmica e mecânica da geleia produzida a partir da polpa.

O estudo reológico de geleias é empregado principalmente para avaliar a sua qualidade e estabilidade, permitindo o desenvolvimento de produtos de maior aceitação

comercial. Nas geleias, a quantidade de sacarose, as proporções e tipos de aditivos geleificantes, a quantidade de polpa, a temperatura e o pH, são os principais fatores que afetam as propriedades reológicas (BASU; SHIVHARE, 2010; BASU; SHIVHARE; SINGH, 2013, GARRIDO; LOZANO; GENOVESE, 2015).

Dessa forma, são encontrados na literatura alguns estudos que avaliam a influência destes fatores nas propriedades reológicas de geleias de manga (BASU; SHIVHARE, 2010), pêssego (*Prunus persica*) (FALGUERA et al., 2010), mirtilo (*Vaccinium myrtillus*) (KECHINSKI et al., 2011) e maçã (TAN et al., 2014; GARRIDO; LOZANO; GENOVESE, 2015). Nestes estudos, as geleias foram avaliadas principalmente quanto ao seu comportamento de fluxo em diferentes condições, apresentando comportamento pseudoplástico e sendo os resultados ajustados nos modelos matemáticos de Herschel-Bulkley e Power Law (este último somente para a polpa de goiaba). Assim, as análises das propriedades reológicas de geleias, aliadas com as análises de textura e sensoriais, contribuem para o desenvolvimento de produtos com características que proporcionem uma maior aceitação do produto pelo consumidor.

TABELA 3 – ESTUDOS DO COMPORTAMENTO REOLÓGICO DE POLPAS DE FRUTAS

(Continua)

Espécie	Objetivo do estudo	Preparo da amostra	Análises reológicas realizadas	Comportamento reológico; modelo matemático mais adequado	Referências
Manga (*Mangifera indica*)	Avaliar a influência do tratamento por alta pressão nas propriedades reológicas da polpa	Polpa triturada e peneirada (poro de 1mm) Tratamento em autoclave 100-400 MPa por 15 ou 30 min, 20 ºC	Reômetro AR 2000 com cilindro concêntrico Curva de fluxo (20 ºC)	Pseudoplástico; Pressões de 100-200 Mpa aumentaram os parâmetros reológicos enquanto que altas pressões diminuíram os mesmos; Modelo Herschel-Bulkley	[1]
Butia (*Butia capitata*)	Analisar a influência da temperatura no comportamento reológico da polpa	Polpa fresca- retirada de casca e sementes em despolpadeira. Polpa peneirada (poro de 1.5 mm)	Reômetro Haake Retovisco modelo RV-20 Curva de fluxo (10-60 ºC)	Pseudoplástico; A viscosidade diminui com o aumento da temperatura; Modelo Herschel-Bulkley	[2]
Açaí (*Euterpe oleraceae* Mart.)	Analisar a influência da temperatura no comportamento reológico da polpa	Polpa congelada, homogeneizada em agitador magnético	Reômetro Carri-Med CSL^2 500 Análise em diferentes temperaturas (10, 25, 40, 55 e 70 ºC) Sensor ranhurado Curva de fluxo Varredura de frequência (0.01-10 Hz)	Pseudoplástico; Efeito de deslizamento da polpa – usa sensor ranhurado para minimizar efeito; Os valores dos módulos G' e G'' diminuem com o aumento da temperatura	[3]
Siriguela (*Spondias purpurea* L.)	Analisar a influência da temperatura no comportamento reológico da polpa	Polpa congelada, homogeneizada	Reômetro AR2000 ex Curva de fluxo (0-80 ºC) Varredura de frequência (0 e 80 ºC)	Pseudoplástico; Modelo Herschel-Bulkley Os módulos G' e G'' foram dependentes da frequência; Comportamento de gel fraco; Análise de temperatura descrita pela equação de Arrhenius	[4]

(Continuação)

Espécie	Objetivo do estudo	Preparo da amostra	Análises reológicas realizadas	Comportamento reológico; modelo matemático mais adequado	Referências
Pequi (*Caryocar coriaceum*)	Analisar a influência da temperatura no comportamento reológico da polpa em diferentes concentrações	Polpa fresca- retirada de casca e sementes em despolpadeira; Polpa concentrada (6, 8, 10 e 12 ºBrix)	Reômetro Brookfield modelo DV-II-PRO Análise do comportamento de fluxo nas temperaturas de 25, 30, 35, 40, 45, 50 ºC	Pseudoplástico A viscosidade aumentou com o aumento de sólidos solúveis; Modelo Mizrahi-Berk; Análise de temperatura descrita pela equação de Arrhenius	[5]
Jabuticaba (*Myrciaria cauliflora*)	Analisar a influência do tamanho de partículas da polpa no comportamento reológico	Polpa fresca – centrifugada (1000 g, 30 min). Partículas de polpa separadas em peneiras de (64-855 μm) e resolubilizadas no sobrenadante centrifugado	Reômetro Carri-Med CSL^2 500 – tensão controlada Curva de fluxo, 25 ºC Varredura de frequência (0,01-10 Hz)	Pseudoplástico O aumento no tamanho de partícula levou ao aumento da viscosidade e a obtenção de géis mais fortes; Modelo Herschel-Bulkley	* [6]
Araçá (*Psidium catteianum*)	Analisar a influência da temperatura no comportamento reológico da polpa	Polpa fresca- retirada de casca e sementes em despolpadeira. Polpa peneirada (poro de 1 mm)	Reômetro Brookfield com cilindro concêntrico Curva de fluxo em temperaturas de 10-60 ºC	Pseudoplástico A viscosidade diminui com o aumento da temperatura; Modelo Herschel-Bulkley Análise de temperatura descrita pela equação de Arrhenius	* [7]
Goiaba (*Psidium guajava*)	Analisar a influência da temperatura no comportamento reológico da polpa em diferentes concentrações	Polpa fresca- retirada de casca e sementes em despolpadeira. Polpa homogeneizada com teor de sólidos solúveis de 8, 10 e 12 ºBrix	Viscosímetro Brookfield modelo RVT. Análise da viscosidade nas temperaturas de 10-60 ºC	Pseudoplástico A viscosidade aumenta com o aumento do teor de sólidos solúveis e diminui com o aumento da temperatura; Modelos Herschel-Bulkley e Mizrahi-Berk. Análise de temperatura descrita pela equação de Arrhenius	* [8]

(Conclusão)

Espécie	Objetivo do estudo	Preparo da amostra	Análises reológicas realizadas	Comportamento reológico; modelo matemático mais adequado	Referências
Goiaba (*Psidium guajava*)	Analisar a influência da temperatura no comportamento reológico da polpa	Polpa	Reômetro Brookfield modelo DV-III Análise do comportamento de fluxo nas temperaturas de 20, 25, 30 e 35 ºC	Pseudoplástico A viscosidade diminui com o aumento da temperatura; Modelo Herschel-Bulkley Análise de temperatura descrita pela equação de Arrhenius	* [9]
Gabiroba (*Campomanesia xanthocarpa*)	Analisar a influência da temperatura no comportamento reológico da polpa	Polpa	Reômetro Brookfield modelo DV-III Análise do comportamento de fluxo nas temperaturas de 20, 25, 30 e 35 ºC	Pseudoplástico A viscosidade diminui com o aumento da temperatura; Modelo Ostwald de Waele Análise de temperatura descrita pela equação de Arrhenius	* [10]
Gabiroba (*Campomanesia xanthocarpa*)	Analisar a influência da temperatura no comportamento reológico da polpa	Polpa	Reômetro HAAKE RS 75 Rheostress Curva de fluxo em diferentes temperaturas (20-60 ºC) Varredura de frequência (0,1-10 Hz)	Pseudoplástico Aumento do comportamento de fluxo com o aumento da temperatura Modelos Herschel-Bulkley e Power Law	* [11]

NOTA: (*) Estudos referentes ao comportamento reológico de algumas espécies de Myrtaceae. [**1**] AHMED; RAMASWAMY; HIREMAYH, 2005; [**2**] HAMINIUK et al., 2006a; [**3**] TONON et al., 2009; [**4**] AUGUSTO et al., 2012 [**5**] SOUSA et al., 2014; [**6**] SATO; CUNHA, 2007; [**7**] HAMINIUK et al., 2006b [**8**] DE OLIVEIRA; ROSSI; DE BARROS, 2011 [**9**] PEREIRA et al., 2012; [**10**] DE OLIVEIRA; ROSSI; DE BARROS, 2011; [**11**] SANTOS et al., 2012. FONTE: O autor (2018).

ARTIGO I

PECTINS FROM THE PULP OF GABIROBA (*Campomanesia xanthocarpa* Berg): STRUCTURAL CHARACTERIZATION AND RHEOLOGICAL BEHAVIOR

Este capítulo é uma reprodução do manuscrito "Pectins from the pulp of gabiroba (*campomanesia xanthocarpa* Berg): Structural characterization and rheological behavior" que será submetido para o periódico Carbohydrate Polymers.

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ABSTRACT

Pectins were extracted from the pulp of gabiroba fruits (*Campomanesia xanthocarpa* Berg) with hot water, giving rise to a fraction GW, comprised of a high-methoxyl pectin (HM) with degree of esterification (DM) of 60%. The pectin fraction showed to be composed mainly of arabinose (54.5%), galacturonic acid (33.5%), galactose (7.6%) and rhamnose (1.6%), with minor amounts of xylose and glucose. The pectins in the fraction GW were characterized by chromatographic and spectroscopic methods indicating the presence of homogalacturonans (HG) and type I rhamnogalacturonans (RG-I). The HG domain represents 31.9% and the RG-I domain 65.3%. Furthermore, GW was submitted to sequential fractionation methods, giving rise to fraction GWP-TEP, characterized by the predominance of homogalacturonan regions, confirmed by ^{1}H-^{13}C heterocorrelated HSQC-NMR spectrum. The rheological behavior of GW was analyzed at 1%, 3% and 5% (w/v) pectin solutions with 0.1 mol L^{-1} NaCl. All samples showed shear thinning behavior. In the oscillatory measurements, the 1% GW showed a liquid-like behavior, while the 3% present a concentrated solution behavior and 5% GW a weak gel behavior.

Keywords: Gabiroba; Myrtaceae family; Pectin; NMR analysis; Rheology.

1 INTRODUCTION

Campomanesia xanthocarpa Berg, popularly known as gabiroba, is a Brazilian native specie of the Myrtaceae family (Barroso, 1978). Gabiroba fruits have high nutritional value and can be consumed *in natura* or used in food formulations (Lisbôa, Kinupp, & Barros, 2011). Gabiroba is the third-richest fruit in vitamin C, with 826 mg 100 g^{-1} (Valor nutricional da guabiroba, 2015), appearing after acerola (*Malpighia emarginata*) with 1.074 mg 100 g^{-1} (Vendramini & Trugo, 2000) and camu-camu (*Myrciaria dubbia*) that presents the highest vitamin C content (2.010 mg 100 g^{-1}) in ripe fruits (Akter, Oh, Eun, & Ahmed, 2011).

The main nutrients in gabiroba fruits are the carbohydrates, representing 7.8-10.2% (Vallilo, Moreno, Oliveira, Lamardo, & Garbelotti, 2008; Andrade, Helm, Mazza, & Mazza, 2012; Santos, Correia, Petkowicz, & Candido, 2012; Barbieri et al., 2017). Among this polysaccharides are the pectins, soluble dietary fibers widely employed in the food industry and in the formulation of cosmetics and pharmaceuticals due to their gelling, emulsifying and stabilizing properties (Voragen, Coenen, Verhoef, & Schols, 2009; Naqash, Masoodi, Rather, Wani, & Gani, 2017).

In recent decades, these biopolymers has attracted a great deal of attention because of their broad spectrum of therapeutic properties, such as immunological (Tamiello, Nascimento, Iacomini, & Cordeiro, 2018), gastroprotective (Cantu-Jungles et al., 2014; Corrêa-Ferreira et al., 2018), antioxidant (Hua, Zhang, Huang, Yi, & Yan, 2014; Klosterhoff et al., 2018) and antitumor (Zong, Cao, & Wang, 2012) effects. Additionally, pectic polysaccharides have also been exploited for drug delivery, gene delivery, wound healing, and tissue engineering due to its biocompatibility, good biodegrability and non-toxicity (Munarin, Tanzi, & Petrini, 2012; Munarin et al., 2015; Noreen et al., 2017).

Pectins are the most complex polysaccharides in the primary cell wall of plants, and are comprised by three main classes of polymers: homogalacturonan (HG), type I rhamnogalacturonan (RG-I) and type II rhamnogalacturonan (RG-II) (Carpita & McCann, 2000; Willats, Knox, & Mikkelsen, 2006; Caffall & Mohnen, 2009). Regarding their monosaccharide composition, pectins are mainly constituted of α-D-GalA, followed by β-D-Gal and α-L-Ara. However, the composition and structure depends on the pectin source, extraction conditions, developmental stages and even the different parts of the fruit (Yapo, 2011).

HG appears as the major component (65%) of pectic polysaccharides. Its structure is formed by (1→4)-linked α-D-galacturonic acid (D-GalA) units that are partially methyl-esterified and sometimes partially acetyl-esterified (Carpita & Gibeaut, 1993; Mohnen, 2008; Yapo, 2011). The degree of methyl esterification (DM) and degree of acetylation (DA) are known to affect the functional properties of pectins and their formed gels (Willats, Knox, & Mikkelsen, 2006; Chan, Choo, Young, & Loh, 2017).

RG-I represent about 20-35% of pectins, presenting a structure of α-D-GalA (1→4)-linked units alternated with α-L-Rha (1→2)-linked units, which may contain neutral side chains, mainly composed of arabinans, galactans and arabinogalactans (Voragen, Coenen, Verhoef, & Schols, 2009; Yapo, 2011).

Nowadays, pectins are mostly produced from citrus peels (56% from lemons, 30% from limes, and 13% from oranges), apple pomace (14%), and a minor portion obtained from sugar beet (Ciriminna, Fidalgo, Delisi, Ilharco, & Pagliaro, 2016). The global market of pectins was valued in more than $850 million in 2013, with a projected annual growth rate ≥ 5%. Despite the projected increase in production, the pectin's demand still exceeds its supply (Ciriminna, Fidalgo, Delisi, Ilharco, & Pagliaro, 2016; Zhang et al., 2018).

In Brazil, in 2017, according to data from the Ministry of Development, Industry and Foreign Trade, approximately 6,000 tons of pectins were exported and around 482 tons were imported (Aliceweb, 2018). Considering that Brazil is the third major worldwide producer of fruits (Clerici & Carvalho-Silva, 2011), other sources, such as native species, can be potentially explored for producing this hydrocolloid.

Despite several studies describing the characterization of pectins deriving from different origins, up to now, only few studies are related to fruits from the Myrtaceae family. The main available information from this family reports data on the chemical and nutritional composition of fruits, such as amount of protein, total carbohydrates, dietary fibers, lipids, vitamins and phenolic compounds (Biegelmeyer et al., 2011; Alezandro, Dubé, Desjardins, Lajolo, & Genovese, 2013; Chaves, Barreto, Reis, & Kadam, 2013). Regarding the chemical structure of pectins, to date, studies have been found presenting only the monosaccharide composition, as reported for gabiroba by Santos, Correia, Petkowicz, & Candido (2012), cambuí (*Myrciaria tenella* Berg) (Vriesmann, Petkowicz, Carneiro, & Belski-Carneiro, 2004), araçá (*Psidium catteleianum)* (Vriesmann, Petkowicz, Carneiro, Costa, & Belski-Carneiro, 2009), Feijoa (*Acca sellowiane*) (Sun-waterhouse, Wang, Waterhouse, & Wadhwa, 2012), and jaboticaba (*Myrciaria cauliflora*) (Moreno, Nascimento, Zielinski, Wosiacki, & Canteri, 2016), presenting as major monosaccharides, arabinose, galacturonic acid and galactose in

different proportions. Some characterization studies of the chemical structure of pectins were performed for goiaba (*Psidium guajava*) (Marcelin, Saunier & Brillouet, 1991; Hua, Zhang, Huang, Yi & Yan, 2014; Zhang et al., 2016), murta (*Ugni molinae Turcz*) (Taboada et al, 2010), myrtle (*Myrtus communis* L.) (Chidouh, Aouadi & Heyraud, 2014) and more recent for jambo (*Syzygium jambos* L.) (Tamiello, Nascimento, Iacomini, & Cordeiro, 2018). Structures of homogalacturonans, type I rhamnogalacturonans and arabinogalactans (AG-I and AG-II) have been reported for those fruits.

The main applications of these polysaccharides, involve their ability to modify the physical properties of the medium in which they are found, to give solutions viscosity or to create cohesive intermolecular networks forming gels (Steffe, 1996). Thus, the knowledge of the rheological properties of pectins contributes to find the best target for these macromolecules in different applications.

In relation to the rheological properties of pectins extracted from fruits belonging to the Myrtaceae family, only a previous work was found for pectins extracted with cold water from the gabiroba pulp, where the solutions at the concentration of 30 g L^{-1} in the presence of calcium presented pseudoplastic behavior and typical network of a gel (Santos, Correia, Petkowicz, & Candido, 2012).

In view of the potential application of pectins, mainly related to its rheological properties, and considering the scarcity of studies on the rheological properties of the pectin obtained from fruits of the Myrtacea family, as *Campomanesia xanthocarpa*, the aim of the present study was to elucidate for the first time, the fine chemical structure of the pectins isolated from the pulp of the gabiroba fruits and evaluate its rheological behavior in solutions**.**

2 MATERIALS AND METHODS

2.1 Plant material

Ripe gabiroba (*Campomanesia xanthocarpa* Berg) fruits were collected from a conservation area in Irati-Paraná/Brazil, located at geographic coordinates of 25° 25' South latitude, 50° 36' West longitude, and 25° 17' South latitude, 50° 30' West longitude. The fruits were selected and washed, the peel and seeds removed in a removing device (Macanuda/ Model SPI-DMJI/2013), and the resulting pulp was frozen at -20 °C for further analyses.

2.2 Extraction and purification of polysaccharides

The gabiroba pulp (1500 g) was thawed and subjected to treatment with 99% ethanol in a boiling water-bath under reflux for 30 minutes giving the alcohol insoluble residues (AIR). The polysaccharides were exhaustively extracted for 4 h (x 7) from the AIR with hot water in a boiling water-bath under reflux. The aqueous extracts were obtained by centrifugation (12.000 x g, 25 min at 4 °C). The volume was reduced under vacuum, followed by treatment with 99% ethanol (3:1, v/v) in order to precipitate the polysaccharides. The precipitated polysaccharides were dialyzed for 48 h against distilled water and the volume was reduced under vacuum, washed with ethanol P.A (x 3) and dried under vacuum. The resulting polysaccharide fraction was named GW (Fig. 1).

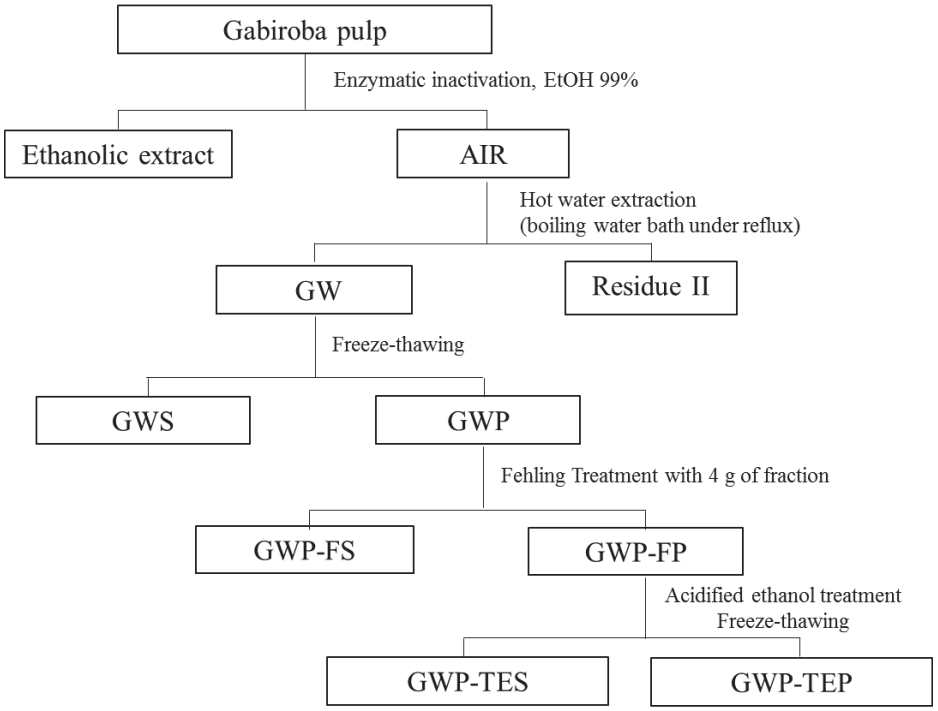

Fig. 1. Scheme of extraction and fractionation of water extracted polysaccharides from the pulp of gabiroba fruits (*Campomanesia xanthocarpa* Berg).

A freeze-thaw treatment was applied to the aqueous fraction GW. In this procedure, the sample was frozen and then allowed to thaw at room temperature followed by centrifugation (12.000 x g, 20 min at 4 °C) (Gorin & Iacomini, 1985), to obtain cold-water soluble (GWS) and insoluble (GWP) fractions. The fraction GWP was subjected to treatment with Fehling's solution (Jones & Stoodley, 1965). A soluble fraction, named GWP-FS and a Cu^{2+} complex precipitate fraction, named GWP-FP were formed. To remove the copper, the

fraction GWP-FP was treated with acidified ethanol according to Hwang, Roshdy, Kontominas & Kokini (1992), freeze-dried and solubilized in water for a second freeze-thaw treatment. A cold-water soluble fraction (GWP-TES) and its respective insoluble fraction (GWP-TEP) were obtained (Fig. 1).

2.3 Monosaccharide composition

The neutral monosaccharides were determined by total acid hydrolysis with 2 mol L^{-1} TFA for 8 h at 100 °C, followed by conversion to alditol acetates by successive $NaBH_4$ reduction (100 °C for 10 min) (Wolfrom & Thompson, 1963a), and acetylation with acetic anhydride (Ac_2O)-pyridine (1:1, v/v, 1 ml) at 100 °C for 30 min (Wolfrom & Thompson, 1963b). The resulting alditol acetates were extracted with $CHCl_3$, and the samples analyzed in a Thermo Scientific Trace GC Ultra gas chromatograph with a mixture of He, N_2 and compressed air as carrier gas at 1 mL min^{-1}, using a DB-225-MS column (0.32 mm internal diameter x 30 m x film thickness 0.25 μm) programmed from 100 °C to 230 °C at a heating rate of 60 °C min^{-1}. The alditol acetates were identified by their profiles, and retention times compared with standards.

Uronic acid contend was quantified by colorimetric *m*-hydroxybiphenyl method, using galacturonic acid as standard (Blumenkrantz & Asboe-Hansen, 1973). The identity of the uronic acid was determined by anion exchange chromatography with pulse amperometric detection (HPAEC-PAD). The fractions were hydrolyzed with 2 mol L^{-1} TFA (8 h, 100 ºC), dryed and washed with methanol (x 3) until total removal of the acid. Samples (1 mg mL^{-1}), were filtered through a membrane of 0.22 μm and injected in a Thermo Scientific Dionex ICS-5000 chromatograph (Thermo Fisher Scientific, USA) with CarboPac PA20 column (3 × 150 mm) using gradient of 0.5 mol L^{-1} NaOH and 1 mol L^{-1} NaOAc as eluent (Nagel, Sirisakulwat, Carle, & Neidhart, 2014) in N_2 atmosphere in a flow of 0.2 mL min^{-1} at 24 ºC. Analyses were carried out in triplicate. Data were collected and analyzed using the ChromeleonTM 7.2 Chromatography Data System software.

2.4 High performance size exclusion chromatography

High performance size exclusion chromatography coupled with multi-angle laser light scattering (DSP-F, Wyatt Technology, Santa Barbara, CA, USA) and refractive index detectors (Waters 2410, Milford, MA, USA) (HPSEC-MALLS-RI) were used to analyze the

homogeneity of the soluble polysaccharides. The chromatography was carried out on a Waters system containing four gel permeation columns packed with Ultrahydrogel® 2000, 500, 250 and 120, connected in series, with exclusion limits of $7x10^6$, $4x10^5$, $8x10^4$ and $5x10^3$ g mol^{-1}, respectively. The flow rate used was 0.6 mL min^{-1} with 0.1 mol L^{-1} sodium nitrite as the mobile phase and 0.2 g L^{-1} sodium azide as a preservative at a temperature of 25 °C. The data was collected and processed by a Wyatt Technology ASTRA software, version 4.70.07.

2.5 Nuclear magnetic resonance (NMR) spectroscopy

Mono-dimensional (^{13}C- and ^{1}H-) and bi-dimensional (HSQC) NMR spectra were acquired at 70 °C on a Bruker AVANCE III 400 NMR spectrometer, operating at 9.5 T, and observing ^{1}H at 400.13 MHz and ^{13}C at 100.61 MHz, equipped with a 5-mm multinuclear inverse detection probe with z-gradient. The samples were solubilized in D_2O and the chemical shifts were expressed as δ (ppm), using the resonances of $-CH_3$ groups of acetone (^{1}H at δ 2.22; ^{13}C at δ 30.20) as internal references. All pulse programs were supplied by Bruker.

2.6 Determination of degree of methyl esterification (DM) and degree of acetylation (DA)

The values of degree of methyl esterification (DM) were determined by ^{1}H-NMR spectroscopy according Grasdalen, Bakoy, & Larsen (1988), integrating the hydrogen areas corresponding to H-1 and H-5 of unesterified α-D-Gal*A*p units and H-1 and H-5 of esterified α-D-Gal*A*p units. Briefly, the fraction GW was deuterium-exchanged three times by freeze-drying with D_2O solution, finally dissolved in D_2O and transferred into 5-mm NMR tube. The ^{1}H-NMR spectra were acquired at 70 ºC with 256 scans on a Bruker AVANCE III 400 NMR spectrometer, operating at 9.5 T, observing ^{1}H at 400.13 MHz. Chemical shifts were expressed as δ (ppm).

The degree of acetylation (DA) was determined by colorimetric method using galactose pentaacetate as standard, at concentration of 0.5-0.6 mg mL^{-1} (Hestrin, 1949), and calculated according Colodel, Bagatin, Tavares, & Petkowicz (2017).

2.7 Preparation of pectin for rheological analysis

For the rheological analyses, the fraction GW was solubilized in an aqueous solution with 0.1 mol L^{-1} NaCl at concentrations of 1, 3 and 5% (w/v), under stirring for 16 h at 25 ºC. The solution was allowed to rest for 2 h prior to the rheological experiments.

2.8 Rheological measurements

Rheological analyses were carried out using a Thermo Scientific Haake Mars II rheometer (Haake GmbH, Germany) coupled to a thermostatized circulating water bath (DC5-Haake K15), regulated by a Peltier (Haake UTM Controller), and kept at 25 °C. The analyzes were performed using a C60/2ºTiL cone and plate geometry with a gap size of 1.0 mm. Flow curves were performed at a shear rate range of 0.01-1000 s^{-1}, for 300 s, at 25 ºC.

Mechanical spectra were obtained in the range of 0.03-10 Hz, at 25 ºC, under stress in the linear viscoelastic region, which was obtained by oscillatory stress sweeps between 0.01 and 100 Pa at constant frequency of 1 Hz. The experiments were carried out in triplicate with mean and standard deviation evaluated by the statistical software Graphpad Prism 5. The samples were placed in the rheometer and kept for 5 min before analysis. A new sample was used for each repetition. The software used for the evaluation of data was HAAKE RheoWin Sofware, version 4.3.

3 RESULTS AND DISCUSSION

3.1 Extraction, fractionation and structural characterization of the pectin from the pulp of gabiroba fruits

The pulp of gabiroba fruits (1500 g), without peel and seeds was treated with EtOH for enzyme inactivation and removal of pigments. The resulting residue was submitted to hot water extraction giving the fraction GW (11.4 g yield) (Fig. 1). GW showed to be composed mainly of arabinose (Ara, 54.5%), followed by galacturonic acid (GalA, 33.5%), galactose (Gal, 7.6%) and rhamnose (Rha, 1.6%), with minor amounts of xylose (Xyl, 1.0%) and glucose (Glc, 0.9%) (Table 1).

In comparison to other fruits of the Myrtaceae family the monosaccharide composition of the fraction GW was similar to that found by Vriesmann, Petkowicz, Carneiro,

Costa, & Belski-Carneiro (2009) for a pectic fraction extracted with water at 25 ºC from the pulp of *Psidium catteleianum* (araçá), which presented Ara (50.3%), Uronic acid (30%) and Gal (10.4%). Else more, GW presented a higher amount of GalA (33.5%), compared to the aqueous fraction extracted at 26 ºC from the gabiroba pulp by Santos, Correia, Petkowicz, & Candido (2012) that showed 16.9% of GalA in its composition.

The pectin fraction (GW) from the gabiroba pulp, showed the highest amount of arabinose (54.5%) in its monosaccharide composition when compared to other pectic polysaccharides obtained from *Myrciaria tenella* Berg (Cambuí) (Vriesmann, Petkowicz, Carneiro, & Belski-Carneiro, 2004), *Myrtus communis* L. (Myrtle) (Chidouh, Aouadi, & Heyraud, 2014), *Ugni molinae* Turcz (Murta) (Taboada et al., 2010), and *Syzygium jambos* L. (Jambo) (Tamiello, Nascimento, Iacomini, & Cordeiro, 2018), which showed in their monosaccharide composition 26.7%, 28.8%, 25.6% and 22.2%, of arabinose, respectively.

Through the monossacaride composition was possible to estimate the molar percentage corresponding to homogalacturonan (HG) and rhamnogalacturonan type I (RG-I) domains of the pectin using the equations:

HG (%) = GalA (%) – Rha (%) and,

RG-I (%) = [GalA (%) – HG(%)] + Rha + Ara + Gal

(M'sakni et al., 2006), respectively. According to these calculations, HG domain represents 31.9% and the RG-I domain 65.3% in fraction GW.

In order to estimate the extension of the neutral side chains, the ratio (Ara+Gal)/Rha was employed (Houben, Jolie, Fraeye, Loey, & Hendrickx, 2011). GW exhibited high (Ara+Gal)/Rha ratio (38.8) indicating that long side chains must be attached in RG-I region.

The chemical structure of GW was investigated by ^{13}C-NMR (Fig. 2) and compared with data available in the literature (Cantu-jungles, Iacomini, Cipriani & Cordeiro, 2017; Klosterhoff et al., 2018; Nascimento, Iacomini, & Cordeiro, 2017). Typical chemical shifts from homogalacturonan were observed at δ 100.1 and 70.5 corresponding to C-1 and C-5 of methylesterified α-D-GalA*p* units, respectively, and the signals at δ 99.3 and δ 71.5 corresponding to C-1 and C-5 of unesterified α-D-GalA*p* units. The remaining assignments of D-GalA*p* ring carbons were assigned at δ 78.6 (*O*-substituted C-4/H-4), δ 68.9 (C-3) and δ 68.1 (C-2). The signals of the C-6 of the unesterified and esterified α-D-GalA*p* units could be assigned at δ 173.8 and δ 170.7, respectively. Signals of methyl and acetyl groups linked to the α-D-GalA*p* units appear at δ 52.8 and δ 20.1, respectively.

Table 1. Monosaccharide composition of pectic polysaccharides obtained from the pulp of gabiroba fruits.

Fraction	Monosaccharide composition (%)[a]					
	GalA[b]	Rha	Ara	Xyl	Gal	Glc
GW	33.5	1.6	54.5	1.0	7.6	0.9
GWS	30.5	0.4	58.4	1.0	8.4	0.5
GWP	40.7	0.9	49.2	0.8	8.3	tr
GWP-FS	20.8	0.4	72.8	0.7	5.3	tr
GWP-FP	65.7	2.0	22.2	1.2	8.1	0.6
GWP-TES	57.2	0.9	34.6	0.4	7.0	-
GWP-TEP	68.1	2.8	8.1	1.8	18.4	0.9

[a] % of peak area of monossaccharide composition relative to the total peak area, determined by GLC.
[b] Uronic acids, determined using the *m*-hydroxybiphenyl method (Blumenkrantz & Asboe-Hansen, 1973), and identified by HPAEC-PAD.
tr - traces.

Due to the presence of esterified α-D-GalA*p* units, the degree of methyl esterification (DM) of GW was determined by ^{1}H-NMR spectroscopy, giving values of 60%, corresponding to a high-methoxyl pectin (HM). HM pectins (degree of methyl esterification, DM > 50%) are characterized by forming gels at acidic pH below 3.5 and in the presence of high concentrations of co-solutes (60-65%), such as sucrose. A low degree of acetylation (DA) was observed for GW (0.02%). A high degree of methyl esterification and a low degree of acetylation are desirable characteristics in the pectins, allowing them to be used as a gelling agent at low pH and high sucrose content. Thus, those polymers can be suitable for use in food formulations (Stephen, Phillips, & Williams, 2006; Chan, Choo, Young, & Loh, 2017).

The ^{13}C-NMR also showed signals at δ 107.6, δ 107.2 and 106.5 attributed to α-L-Ara*f* (C-1). Signals for the (1→4)-linked-β-D-Gal*p* units were showed at δ 104.3 (C-1), δ 71.9 (C-2), δ 73.4 (C-3), δ 77.6 (*O*-substituted C-4), δ 74.5 (C-5) and δ 60.8 (C-6). α-L-Rha*p* units were also observed and the signal at δ 16.6 attributed to C-6 of type I rhamnogalacturonan (RG-I) from hairy regions in the pectin chain (Fig 2). All signals were assigned by comparison with the literature (Petkowicz, Vriesmann, & Williams, 2017; Colodel, Bagatin, Tavares & Petkowicz, 2017; Nascimento, Iacomini, & Cordeiro, 2017).

Thus, the results of monosaccharide composition and NMR indicate the presence of homogalacturonans (HG), type I rhamnogalacturonans (RG-I) and/or arabinogalactans (AG), arabinans and galactans in the fraction GW. These results were in agreement with the outcomes of earlier studies where pectins were also obtained by aqueous extraction from other

fruits that also belong to the Myrtaceae family, such as Myrtle (*Myrtus communis* L.) (Chidouh, Aouadi, & Heyraud, 2014), Murta (*Ugni molinae* Turcz) (Taboada et al., 2010), Guava (*Psidium guajava* Linn) (Hua, Zhang, Huang, Yi, & Yan, 2014), Jaboticaba (*Myrciaria cauliflora*) (Moreno, Nascimento, Zielinski, Wosiacki, & Canteri, 2016) and Jambo (*Syzygium jambos* L.) (Tamiello, Nascimento, Iacomini, & Cordeiro, 2018), which presented pectic fractions constituted by HG, RG-I, AG-I and AG-II shown by NMR analyzes.

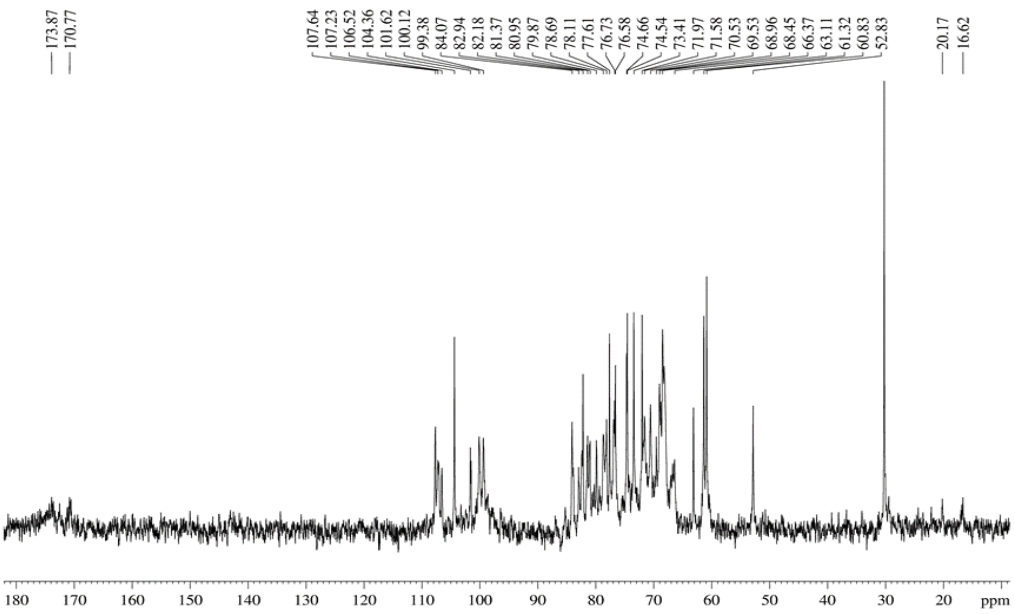

Fig. 2. ^{13}C NMR spectrum of the fraction GW from the pulp of gabiroba fruits, obtained at 70 °C in D_2O (chemical shifts are expressed in δ, ppm).

The homogeneity of GW was determined by high performance size exclusion chromatography (HPSEC) (Fig. 3A), which gave rise to a heterogeneous elution profile with three main peaks showed by RI detector at 42 (I), 48 (II) and 53 (III) min, indicating the presence of different populations of pectins, in this fraction. The light scattering (LS) chromatogram, shows one peak in 40 min corresponding to the largest molar mass and accordance to the first peak detected by refraction index (RI). The molar mass distribution showed a range of molar mass between 10,000 g mol^{-1} and 1,000.000 g mol^{-1} for the fraction GW.

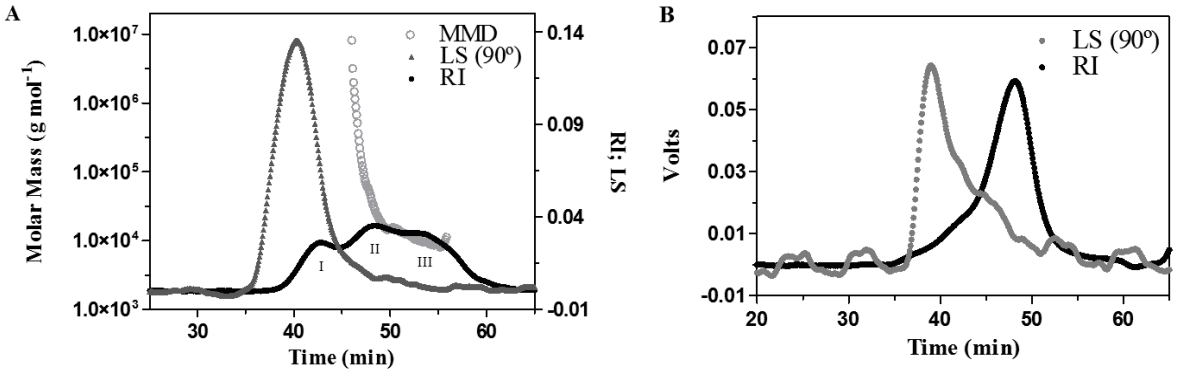

Fig. 3. HPSEC elution profile. (**A**) fraction GW (B) fraction GWP-TEP obtained from the pulp of gabiroba fruits. Molar mass distribution (MMD), light scattering (LS 90º) and refractive index (RI).

In order to fractionate the different polymers from GW it was submitted to freeze-thawing process giving rise to a cold-water soluble fraction (GWS; 3.8 g yield) and a cold-water insoluble fraction (GWP; 7.26 g yield). The GWS and GWP fractions showed similar monosaccharide composition (Table 1). Therefore, we selected the GWP fraction, which presented higher yield (7.26 g), to continue the fractionation. GWP showed higher amount of GalA (40.7%) and lower amount of Ara (49.2%) compared to the original GW fraction (GalA, 33.5%; Ara, 54.5%). Part of GWP fraction (4 g) was submitted to the treatment with Fehling's solution, giving rise to GWP-FS (Fehling supernatant) and GWP-FP (Fehling precipitate). The fraction GWP-FS showed to be mainly composed of Ara (72.8%), followed by GalA (20.8%), Gal (5.3%) and Rha (0.4%) (Table 1), and presented a heterogeneous profile in HPSEC-MALLS-RI with the presence of three peaks in the chromatogram of RI (data not shown), as observed for the fraction GW (Fig. 3).

Monosaccharide composition analysis revealed that GWP-FP (2.03 g yield), was comprised mainly of GalA (65%), Ara (22.2%), Gal (8.1%) and a lower amount of Rha (2.0%) (Table 1). According to the literature, HG rich chains may precipitate using Fehling's solution (Cantu-Jungles et al., 2014; Nascimento et al, 2015; Leivas, Iacomini, & Cordeiro, 2016; Nascimento, Iacomini, & Cordeiro, 2017). Therefore, as indicated by the monosaccharide composition of GWP-FP, precipitated after treatment with Fehling's solution, suggesting that mainly a HG is present in this fraction, which formed a complex with Cu^{2+}.

Therefore, the fraction GWP-FP was further subjected to a treatment with acidified ethanol to remove the remaining Cu^{2+} in this fraction from the treatment with Fehling's

solution. After treatment with acidified ethanol, the fraction GWP-FP was freeze-dried, solubilized in water and a new freeze-thawing process was done in order to isolate the homogalacturonan present in the fraction. Therefore, fractions GWP-TES, soluble in cold-water (0.18 g yield) and GWP-TEP (0.16 g yield) insoluble in cold-water were obtained. The fraction GWP-TES presented a higher proportion of Ara (34.6%) in its composition compared to GWP-TEP (8.1%) (Table 1). The relative amount of HG and RG-I was estimated for fraction GWP-TES, which showed to be composed of 56.3% HG and 43.4% RG-I. This fraction also presented a long side chain (46.2), according to the ratio (Ara+Gal)/Rha.

The homogeneity of the fraction GWP-TEP was analyzed by HPSEC (Fig. 3B). The elution profile detected by the RI, showed a main peak eluting at retention time of 48 min demonstrating that the fractionation process adopted, using the Fehling's solution treatment followed by freeze-thawing was efficient. The monosaccharide composition of GWP-TEP showed this fraction to be mainly composed of GalA (68%), followed by Gal (18.4%), Ara (8.1%) and Rha (2.8 %). In this fraction, the percentage of HG increased to 65.3%, twice more than that observed for the original GW fraction (31.9%), while the RG-I domain decrease to 32.1%. These results were very similar to those found for watermelon (*Citrullus lanatus*) pectins (65.8% of HG and 29% of RG-I) described by Petkowicz, Vriesmann & Williams (2017). Low (Ara+Gal)/Rha ratios were observed for GW-TEP with a value of 9.4, indicating short side chains attached to the RG-I. This ratio was similar to that observed for the watermelon pectin (8.0) (Petkowicz, Vriesmann & Williams, 2017) and to murta (*Ugni molinae Turcz*) pectin (8.1) observed by Taboada et al. (2010).

The results obtained, suggest the predominance of HG regions in the fraction GW-TEP, which was confirmed by the analysis of ^{1}H-^{13}C heterocorrelated HSQC-NMR spectrum (Fig. 4). The highest intensity chemical shifts in the spectrum (Fig. 4) were attributed to C-1/H-1 at δ 100.4/5.09, C-2/H-2 at δ 68.9/3.77, C-3/H-3 at δ 68.9/4.0, C-4/H-4 at δ 79.0/4.48 and C-5/H-5 at δ 71.0/5.0 from α-D-GalA*p* (1→4)-linked units. The signal corresponded to C-6 of unesterified α-D-GalA*p* (1→4)-linked units was observed in ^{13}C-NMR at δ 172.8 (data not shown), confirming the presence of a HG in fraction GWP-TEP.

Signals with lower intensity were also observed in the spectrum showing the C-1/H-1 at δ 99.7/4.99 and C-6/H-6 of α-L-Rha*p* (1→2)-linked units at δ 16.05/1.25 ppm and 4-*O*-substituted α-L-Rha*p* (1→2)-linked units at δ 16.05/1.32 ppm, attributed to type I rhamnogalacturonan (RG-I). Other signals with lower intensity present in the HSQC spectrum, C-1/H-1, C-2/H-2, C-3/H-3, C-4/H-4, C-5/H-5 and C-6/H-6 at δ 104.0/4.62, δ 72.8/3.62, δ 68.5/3.92, δ 78.3/ 4.15, δ 75.3, 3.69 and δ 60.8/3.80 ppm were attributed to

β-D-Gal*p* (1→4)-linked units (Fig. 4). These signals can be attributed to the main chain of galactans or type I arabinogalactan (AG-I), which can be substituting the RG-I main chain. Although the monosaccharide composition presented an amount of 8.1% of Ara, the signals of α-L-Ara*f* units could not be observed in the HSQC-NMR spectrum of GWP-TEP. All signal assignments were in agreement with published data for other fruits as Cubiu (*Solanum sessiflorum* D.) (Colodel, Bagatin, Tavares & Petkowicz, 2017), cacao (*Theobroma cacao* L.) (Vriesmann & Petkowicz, 2017), açaí berries (*Euterpe oleraceae*) (Cantu-jungles, Iacomini, Cipriani & Cordeiro, 2017) and sweet peppers (*Capsicum annum*) (Nascimento, Iacomini, & Cordeiro, 2017). For the pectin structures from the gabiroba pulp, this is the first work that characterizes these chemical structures, contributing to the knowledge of this species.

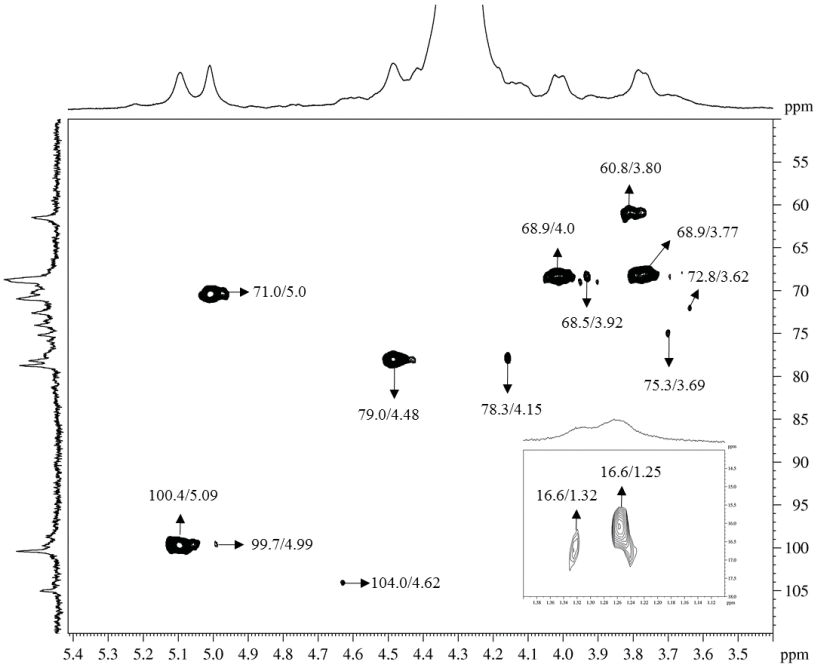

Fig. 4. $^{1}H/^{13}C$ HSQC-NMR correlation map of GWP-TEP fraction. Sample was dissolved in deuterium oxide (D_2O) and data collected at probe temperature at 70 ºC. Chemical shifts are expressed in δ, ppm.

3.2 Rheological analyses of fraction GW

According to the determination of the methyl esterification degree, obtained from integration of hydrogen areas by mono-dimensional ^{1}H-NMR, the pectin extracted from the gabiroba pulp (fraction GW) presents a DM of 60%, corresponding to a high-methoxyl pectin (HM). Due to the wide application of HM pectins, the flow behavior of 1%, 3% and 5% (w/w) solutions of gabiroba pectins were investigated at 25 ºC, using 0.1 mol L^{-1} NaCl to

minimize the charge effect on rheological analyses. Negative charges along the polymer chain (carboxyl groups corresponding mainly to galacturonic acid units) make chain conformation dependent on salinity (Iagher, Reicher & Ganter, 2002).

In the flow curve (Fig. 5A) it was observed an increase of shear stress for 0.1 mol L^{-1} NaCl GW fractions, in accordance with the increase of pectin solution concentration, in response to the increase of shear rate. This behavior occurred by the fact that pectin molecules are dispersed in solution, and are too far apart to interact with one another. At shear rate of 600 s^{-1}, the shear stress was 1.58 Pa, 5.02 Pa, 9.87 Pa, for GW fractions at 1%, 3% and 5%, respectively. So, when the concentration is increased, the intermolecular distances between the pectin molecules decrease, facilitating intermolecular interactions such as hydrogen bonding (Guimarães, Coelho Júnior, & Rojas, 2009; Chan, Choo, Young, & Loh, 2017). This behavior can be observed for pectin from other fruits, as mango (*Mangifera indica* L.) pulp (Iagher, Reicher & Ganter, 2002), apple (*Malus domestica*) pomace (Min et al., 2011), tamarillo fruit (*Solanum betaceum*) pulp (Nascimento, Simas-Tosin, Iacomini, Gorin, & Cordeiro, 2016) and grapefruit (*Citus paradise*) peel (Wang et al., 2016).

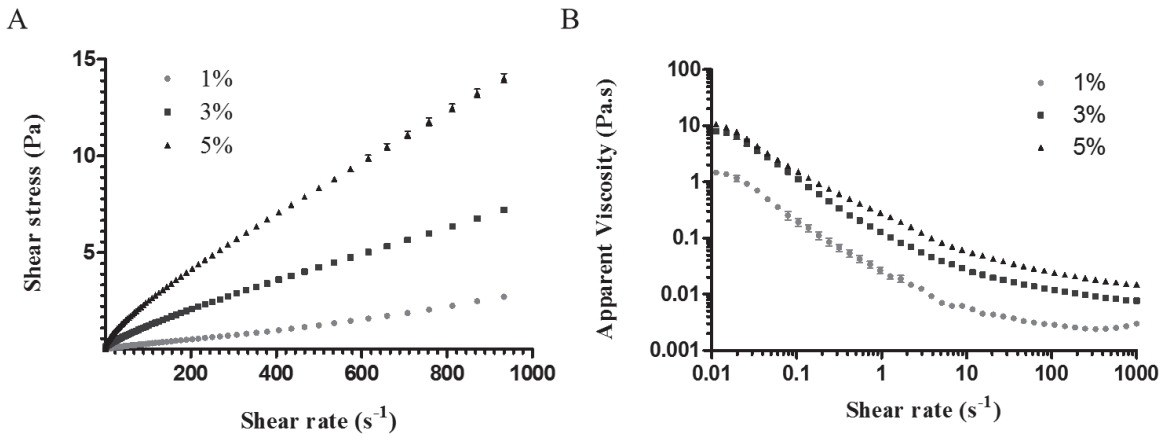

Fig. 5. Influence of shear rate on the flow curve (**A**) and viscosity curve (**B**) of GW (0.01 – 1000 s^{-1}) at 25 ºC.

The viscosity curve of the samples is presented in Fig. 5B as a function of shear rate. The shear thinning behavior was observed for all samples as the apparent viscosity decreased with the shear rate increase. In Fig. 5B it is also shown that the viscosity of the samples increaseed according to its concentration. Thus, in the shear rate at 1 s^{-1}, the apparent viscosity values of GW 1%, 3% and 5% pectin solutions were 0.02, 0.13, and 0.28 Pa, respectively. In the literature, studies reporting the observation of an increase in the viscosity with the increase in pectin concentration are related to the increase of pseudoplasticity of the

solution (Muhammad, Mohd.Zahari, Gannasin, Mohd.Adzahan, & Bakar, 2014; Nascimento, Simas-Tosin, Iacomini, Gorin, & Cordeiro, 2016; Sato, Oliveira, & Cunha, 2008; Muhammad, Mohd.Zahari, Gannasin, Mohd.Adzahan, & Bakar, 2014; Sousa, Nielsen, Armagan, Larsen, & Sorensen, 2015).

Oscillation measurements were carried out in order to characterize the viscoelastic behavior of 1%, 3% and 5% GW pectin solutions. Mechanical spectra (Fig. 6) show elastic modulus (G') and viscous modulus (G") reported as a function of frequency.

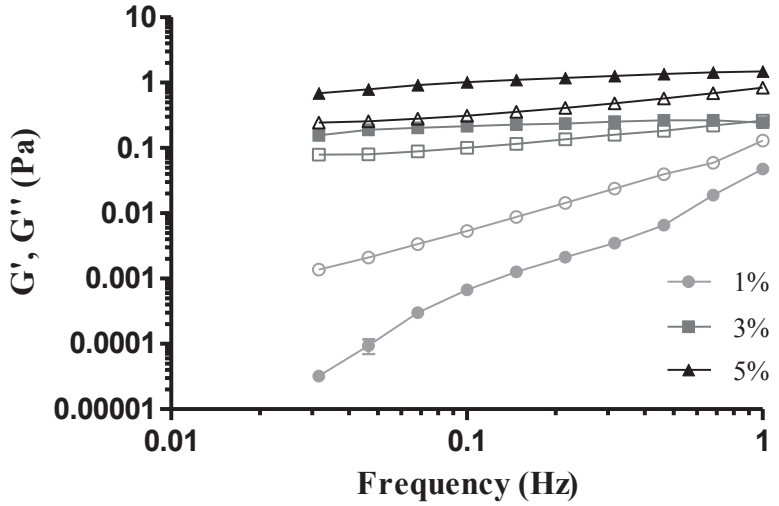

Fig. 6. Mechanical spectra at 25 ºC of GW at 1%, 3%, 5% (w/v) pectin solutions with 0.1 mol L^{-1} NaCl. Elastic modulus (G', full symbols) and viscous modulus (G'', open symbols).

In the mechanical spectra (Fig. 6) it can be observed that in the GW 1% pectin solution, G'' was higher than G' over the analyzed frequency, with both moduli increasing with the increase of the frequency, showing a liquid-like behavior. This behavior has been observed for pectins dispersed in water, as for tamarillo pectin (Nascimento, Simas-Tosin, Iacomini, Gorin, & Cordeiro, 2016) and mango pectin (Iagher, Reicher & Ganter, 2002). Increasing the pectin concentration in solution, was observed that the GW 3% present a concentration solutions behavior observed by crossing of the moduli in frequencies above 1 Hz, while the GW 5% pectin solution showed a typical weak gel behavior. Colodel, Bagatin, Tavares & Petkowicz (2017) also showed that a 5% cubiu pectin solution presented a weak gel behavior, occurring the breaking of the three-dimensional structure in frequencies above 0.46 Hz. Contrary of these results, Vriesmann & Petkowicz (2013) observed that cocoa pectin in a 5% aqueous solution presented a liquid behavior in the frequency of 0.01 to 10 Hz.

The knowledge of the chemical structure and the rheological properties of the pectin obtained from the gabiroba pulp, can add commercial value to the fruit, promoting its use for different applications. Furthermore, is possible to suggest its use in food formulations as a gabiroba jam, where gabiroba pectin could replace commercial citrus pectin, usually used. However, some analyzes still need to be done regarding the gelation process of these pectins, especially in the presence of a co-solute as sucrose, in order to have more knowledge and improve its rheological properties to expand its potential application.

4 CONCLUSION

In the present study, pectic polysaccharides were extracted from gabiroba pulp using hot water. The analyses of monosaccharide composition, homogeneity and nuclear magnetic resonance 1D and 2D, allowed characterizing the pectins present in the pulp of gabiroba fruits. The results indicate the presence of homogalacturonans (HG) and type I rhamnogalacturonans (RG-I) in the fraction GW, and mainly homogalacturonans in the fraction GWP-TEP. The rheological analysis showed a shear thinning behavior for the pectin solutions at different concentrations (1%, 3% and 5%) in the presence of 0.1 mol L^{-1} NaCl. In addition, an increase in viscosity was observed according to the increase of pectin concentration in the solutions. In the oscillatory rheological analysis, the pectin solution at 1% concentration showed viscoelastic behavior with liquid characteristics, while 3% present a concentration solutions behavior observed by crossing of the moduli in frequencies above 1 Hz and 5% GW solutions showed a typical weak gel behavior. It was observed that the values of the G' and G" modules also increased in response to the increase of the pectin concentration in the solutions. Thus, for the first time, the fine chemical structure of pectin from the gabiroba pulp was studied, contributing to the knowledge of this native Brazilian fruit, and adding information about the Myrtacea family.

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ARTIGO II

EXTRACTION, PURIFICATION AND STRUCTURAL CHARACTERIZATION OF A GALACTOGLUCOMANNAN FROM THE GABIROBA FRUIT (*Campomanesia xanthocarpa* Berg), MYRTACEAE FAMILY

Este capítulo é uma reprodução do artigo intitulado "**Extraction, purification and structural characterization of a galactoglucomannan from the gabiroba fruit (*Campomanesia xanthocarpa* Berg), Myrtaceae family**" publicado em 15 de outubro de 2017 no periódico Carbohydrate Polymers (volume 174, página 887-895; website: https://doi.org/10.1016/j.carbpol.2017.07.015).

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ABSTRACT

In this study, we isolated and structurally characterized, for the first time, a galactoglucomannan (GGM) from the pulp of gabiroba, a Myrtaceae family species. The HPSEC-MALLS-RI analysis showed a homogeneous polysaccharide with molar mass of 25,340 g mol^{-1}. The monosaccharide composition showed that the GGM consisted of Man:Glc:Gal in a molar ratio of 1:1:0.6. Methylation and 1D and 2D NMR analyses suggested that the main chain of the GGM consisted of β-D-Glc*p* and β-D-Man*p* units (1→4)-linked. The α-D-Gal*p* substitutions occur mainly at O-6 position of β-D-Man*p* units. The glycosidic linkages of the GGM were evident by the presence of the characteristic signals of 4-*O*-substituted residues at δ 78.6/3.69 for both β-D-Glc*p* and β-D-Man*p*. Furthermore, the O-6 substitutions for both β-D-Glc*p* and β-D-Man*p* units were confirmed by signals at δ 67.1/4.00 and 3.93. The interglycosidic correlations, obtained through the analysis of the HMBC spectrum, further confirm the structure.

Keywords: *Campomanesia xanthocarpa* Berg; Extraction polysaccharide; Gabiroba pulp; Hemicelullose; Galactoglucomannan; NMR analysis.

1 INTRODUCTION

The plant cell wall is a highly complex and dynamic structure made up of cellulose, hemicelluloses, pectins, lignin, and proteins (Burton, Gidley, & Fincher, 2010; McNeil, Darvill, Fry, & Albersheim, 1984). Among the polysaccharides that constitute the cell wall, cellulose and the hemicelluloses present a β-(1→4)-linked backbone. The main hemicelluloses are xyloglucans, xylans, mannans, glucomannans and galactoglucomannans (Scheller & Ulvskov, 2010). These structures have an important biological role as they contribute to strengthening the cell wall by interaction with the cellulose microfibrils (Scheller & Ulvskov, 2010).

Xyloglucan (XyG) is the major hemicellulose in the primary cell wall of dicotyledones; it is involved in the growth and expansion of cells (Hayashi, 1989). In addition, XyGs, because of their ability to interact with cellulose through hydrogen bonds, have been well characterized as an inter-molecular tether between cellulose fibrils (Hayashi, 1989; Scheller & Ulvskov, 2010).

Unlike xyloglucans, the structure of the galactoglucomannans (GGMs) has been scarcely characterized except in the case of wood; neither has a clear role been established for them within the overall architecture of the wall (Schröder et al., 2001). What is already known is the capacity of GGMs to bind to cellulose microfibrils. Based on this, it is assumed that GGMs, as well as XyGs, may have a role in the control of cell expansion (Nara, Ito, Kato, & Kato, 2004).

GGMs have been characterized in different organisms: aquatic mosses (Geddes & Wilkie, 1972), ferns (Bremner & Wilkie, 1971), lichens (Woranovicz, Pinto, Gorin, & Iacomini, 1999), fungi (Ahrazem et al., 2006), gymnosperms (Hannuksela & Du Penhoat, 2004; Xu et al., 2010) and angiosperms (Magnoliophyta) (Guo et al., 2012; Schröder et al., 2001; Sims, Craik, & Bacic, 1997). In gymnosperms, present mostly in spruce wood, GGM appear as the predominant hemicellulose. Spruce GGM consists of a linear backbone of randomly distributed (1→4)-linked β-D-mannopyranosyl (Man*p*) and (1→4)-linked β-D-glucopyranosyl (Glc*p*) units, with single (1→6)-linked α-D-galactopyranosyl (Gal*p*) units attached to the main chain, preferably on the β-D-Manp units. The β-D-Manp units are naturally acetylated at positions C-2 or C-3 (Hannuksela & Du Penhoat, 2004; Willför et al., 2003). Spruce GGMs have been extensively studied; they have shown potential as a novel natural material and hydrocolloid that can be used as food packaging barriers, bioactive

oligosaccharides, dietary fibers and health-promoting agents (Willför, Sundberg, Tenkanen, & Holmbom, 2008). The use of GGMs as basis for hydrogels and films has also been investigated (Hartman, Albertsson, & Sjöberg, 2006; Mikkonen, Heikkilä, Helén, Hyvönen, & Tenkanen, 2010). Naturally acetylated and deacetylated spruce GGMs have also been reported to be useful as biological-response modifiers and therapeutic agents (Ebringerová et al., 2008).

Regarding the GGMs from angiosperms (Magnoliophyta), their presence in the cell wall of the fruits of *Actinidia deliciosa* (kiwifruit) (Schröder et al., 2001) and *Malus domestica* (apple) (Nara, Ito, Kato, & Kato, 2004) have been reported. The GGMs extracted from the pulp of these fruits were characterized by a backbone of β-D-Glc*p*-(1→4)-linked and β-D-Man*p*-(1→4)-linked, some of which were substituted by single D-Gal*p* or by D-Gal*p* disaccharide chains at the O-6 position (Nara, Ito, Kato, & Kato, 2004). The reported ratio of Gal:Glc:Man varies depending on the plant source and the stage of the tissue development (Schröder et al., 2001). However, by our knowledge, no information is available about the structural composition of hemicelluloses such as GGM extracted from the pulp of fruits of the Myrtaceae family.

The Myrtaceae family (Barroso, 1978), is comprises of 145 genera and 5,970 known species spread across the world (The plant list - Myrtaceae, 2013), among which the Brazilian native species is called *Campomanesia xanthocarpa* Berg. *C. xanthocarpa* is also popularly known as gabiroba (Sarmento, Carolina, & Santos, 2012). Its fruits can be consumed in natura or used in food formulations (Kinupp & Barros, 2008). Compounds from C. xanthocarpa leaves and the lipophilic extract of their fruits are also known for their medicinal properties: anti-inflammatory and antioxidant (Klafke et al., 2016), antiulcerogenic (Markman, Bacchi, & Kato, 2004), and reduction of cholesterol levels and obesity (Biavatti et al., 2004).

Considering the scarcity of studies on the polysaccharide from Brazilian native plants such as *C. xanthocarpa* (gabiroba), and in view of the potential application of the different hemicelluloses as already described, the aim of the present study was to elucidate the fine chemical structure of the galactoglucomannan present in the pulp of *C. xanthocarpa* Berg (gabiroba) fruits. The knowledge on the occurrence, purification and chemical structure of its polysaccharides may open new perspectives for the exploitation of *C. xanthocarpa* fruits in different biological and biotechnological applications.

2 MATERIALS AND METHODS

2.1 Plant material

Ripe gabiroba fruits were collected from conservation units in Irati-Paraná/Brazil, located at geographic coordinates of 25° 25' south latitude, 50° 36' west longitude, and 25° 17' south latitude, 50° 30' longitude west. The fruits were selected and washed, the peel and seeds removed in a Macanuda removing device (Model SPI-DMJI/2013), and the resulting pulp stored at -20°C. A moisture analyzer (Model-OHAUS MB25) was used to evaluate the moisture content of the pulp.

2.2 Extraction and purification of polysaccharides

The pulp (1500 g) was thawed and subjected to treatment with 99% ethanol in a boiling water-bath under reflux for 30 minutes, giving the residue I. The residue I was subjected to hot water extraction for 4 hours giving fraction GW and residue II. Then the residue II obtained after centrifugation was submitted to a 5% citric acid extraction giving GCA fraction and residue III. The hemicelluloses were extracted from the residue III by successive alkaline treatment (2 mol L^{-1} and 4 mol L^{-1} NaOH) (Figure 1). Successive alkaline extractions with 2 mol L^{-1} NaOH (x 4) and 4 mol L^{-1} NaOH (x 2) in the presence of $NaBH_4$ were performed in a mechanical blender; after each round of extraction, centrifugation (12.000 x g, 20 min at 4 °C) was carried out to separate the resulting extracts from the respective residues. The alkaline extracts were then neutralized with acetic acid and dialyzed for 48 hours against distilled water. The volume was reduced under vacuum, followed by treatment with 99% ethanol (3:1, v/v) in order to precipitate the polysaccharides. The precipitated polysaccharides were then washed with ethanol P.A (x 3) and dried under vacuum. The resulting polysaccharide fractions were named GA2M and GA4M, in reference to the alkaline extraction with 2 mol L^{-1} NaOH and 4 mol L^{-1} NaOH, respectively.

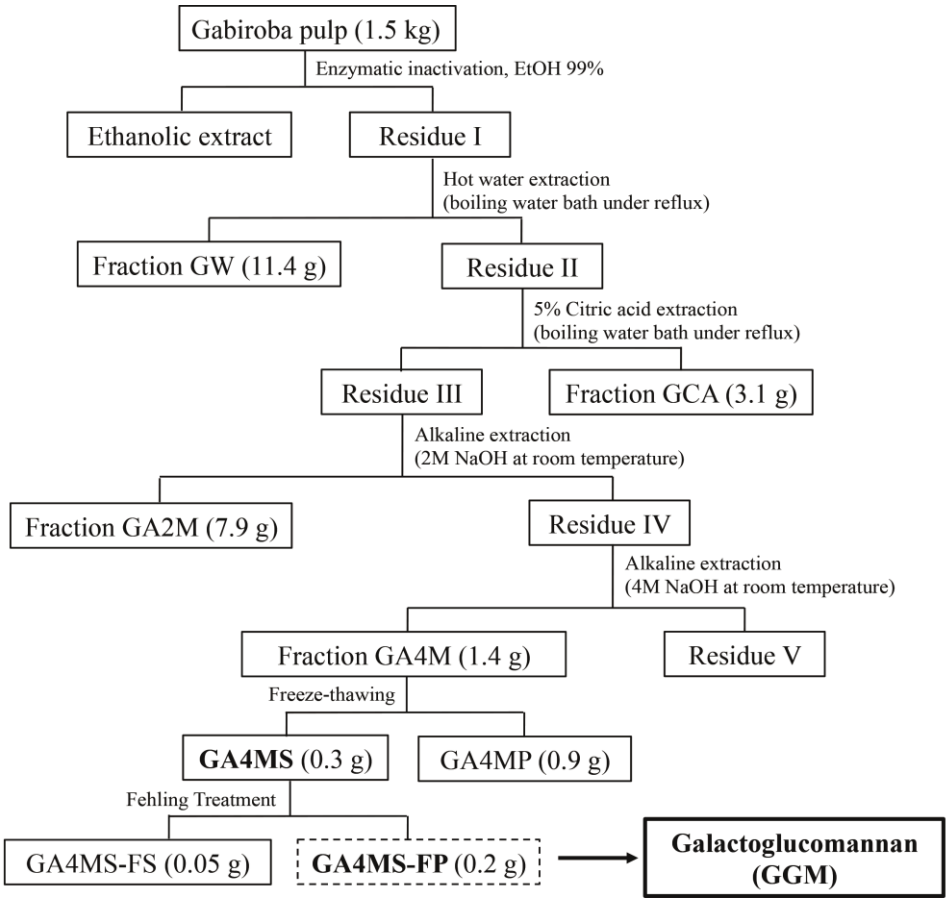


Fig. 1. Scheme of extraction and fractionation of polysaccharides from the pulp of gabiroba fruits.

A freeze-thaw treatment was applied to the alkaline fraction GA4M, to obtain a cold-water soluble fraction (GA4MS) (Fig. 1). In this procedure, the sample was frozen and then thawed at room temperature (Gorin & Iacomini, 1985). The cold-water insoluble polysaccharide fraction GA4MP was recovered by centrifugation (12. 000 x g, 20 min at 4 °C), while the soluble fraction GA4MS was subjected to treatment with Fehling's solution (Jones & Stoodley, 1965). As a result, a soluble fraction named GA4MS-FS and a Cu^{2+} complex insoluble fraction named GA4MS-FP were formed.

2.3 Monosaccharide composition

The neutral monosaccharides were determined by total acid hydrolysis with 2 mol L^{-1} TFA for 8 hours at 100 °C, followed by conversion to alditol acetates by successive $NaBH_4$ reduction (100 °C for 10 minutes) (Sassaki et al., 2008; Wolfrom & Thompson, 1963a), and acetylation with acetic anhydride (Ac_2O)-pyridine (1:1, v/v, 1 ml) at 100 °C for 30 minutes (Wolfrom & Thompson, 1963b). The resulting alditol acetates were extracted with

$CHCl_3$, and the samples analyzed by gas chromatography mass spectrometry (GC-MS) using a Varian Saturn 4000R model 3800 gas chromatograph linked to a Varian Ion-Trap 2000R mass spectrometer, with He as carrier gas. For the quantitative analysis, we used a capillary column (30 m x 0.25 mm, 0.25 μm) of VF-5ms, held at 250 °C. During injection, the program was heated at 100 °C for 3 minutes, followed by heating at 10 °C/min until 220 °C for 3 minutes, 250 °C for 3 minutes, and 280 °C for 8 minutes. The alditol acetates were identified by their profiles, and retention times compared with standards. Uronic acid was estimated by the *m*-hydroxydiphenyl method (Blumenkrantz & Asboe-Hansen, 1973).

2.4 High performance size exclusion chromatography coupled to multidetectors (HPSEC-MALLS-RI)

High performance size exclusion chromatography coupled with multi-angle static laser light scattering (DSP-F, Wyatt Technology, Santa Barbara, CA, USA) and refractive index detectors (Waters 2410, Milford, MA, USA) (HPSEC-MALLS-RI) was used to analyze the homogeneity and average molar mass (M_W) of the soluble polysaccharides.

The log plot M_w versus elution time was calculated from the ratio $Kc / R_\theta = 1 / M_w + 2A_2c$ (Wyatt, 1993; Zimm, 1948), where **K** is the optical constant appropriate for vertically polarized incident light and the polymer solutions used, **c** is the concentration of polymer (g mL^{-1}), $\mathbf{R_\theta}$ is the excess Rayleigh laser light scattering ratio (cm^{-1}), $\mathbf{M_w}$ is the average molecular mass by weight and $\mathbf{A_2}$ is the second virial coefficient. The specific refractive index increment (*dn/dc*), which is defined as the slope of the dependence of refractive index of polymer solution of its concentration, was measured by using a refractive index detector (RI). A *dn/dc* value of 0.146 mL g^{-1} for GA4MS-FP was calculated and used. The chromatography was carried out on a Waters system containing four gel permeation columns packed with Ultrahydrogel® 2000, 500, 250 and 120, connected in series, with exclusion limits of $7x10^6$, $4x10^5$, $8x10^4$ and $5x10^3$ g mol^{-1}, respectively. The flow rate used was 0.6 mL min^{-1} with 0.1 mol L^{-1} sodium nitrite as the mobile phase and 0.2 g L^{-1} sodium azide as a preservative at a temperature of 25 °C. The data was collected and processed by a Wyatt Technology ASTRA software, version 4.70.07.

2.5 Nuclear magnetic resonance (NMR) spectroscopy

Mono-dimensional (^{13}C- and ^{1}H-) and bi-dimensional (HSQC-DEPT, coupled HSQC and HMBC) NMR spectra were acquired at 70 °C on a Bruker AVANCE III 400 NMR spectrometer, operating at 9.5 T, and observing ^{1}H at 400.13 MHz and ^{13}C at 100.61 MHz, equipped with a 5-mm multinuclear inverse detection probe with z-gradient. The samples were solubilized in D_2O and the chemical shifts were expressed as δ (ppm), using the resonances of -CH_3 groups of acetone (^{1}H at δ 2.22; ^{13}C at δ 30.20) as internal references. All pulse programs were supplied by Bruker.

2.6 Methylation analysis of the polysaccharides

The fraction GA4MS-FP was partially-*O*-methylated according to the method of (Ciucanu & Kerek, 1984), using powdered NaOH in Me_2SO/MeI. The per-*O*-methylated products were hydrolyzed with 72% (w/w) H_2SO_4 (0.5 mL) at 0 °C for 1 hour, followed by dilution to 8% (Saeman, Moore, Mitchell, & Millet, 1954), being maintained at 100 °C for 16 hours, and then neutralized with $BaCO_3$. The resulting mixture of partially *O*-methylated monosaccharides, recovered after filtration to remove $BaCO_3$, was reduced with $NaBH_4$ (16 hours at room temperature) and acetylated with Ac_2O:pyridine (1:1, v/v, 1 ml) at 100 °C for 30 minutes. The partially *O*-methylated alditol acetates were then analyzed by GC-MS. A capillary column (30 m x 0.25 mm, 0.25 μm) of VF-5ms, held at 250 °C, was used for the analysis. During injection, the program was heated at 100 °C for 3 minutes, followed by heating at 10 °C min^{-1} until 220 °C for 3 min, 250 °C for 3 min and 280 °C for 8 min. The partially O-methylated alditol acetates were identified by their typical electron impact breakdown profiles and retention times (Sassaki, Gorin, Souza, Czelusniak, & Iacomini, 2005).

3 RESULTS AND DISCUSSION

3.1 Sequential extraction and fractionation of the hemicelluloses from the pulp of gabiroba fruits and their composition

The pulp of gabiroba fruits, after the removal of the peel and seeds, presented a moisture content of 82.3 % ± 0.8. The fresh pulp (1500 g) was treated with EtOH for enzyme

inactivation and removal of pigments. The resulting residue was submitted to successive extractions with hot water, 5% citric acid, 2 mol L^{-1} and 4 mol L^{-1} sodium hydroxide solutions, giving the polysaccharides fractions GW, GCA, GA2M, and GA4M, respectively (Fig. 1).

The monosaccharide analyses (Table 1, Fig. S1) revealed that the water-soluble fraction GW (11.4 g yield) was comprised mainly of uronic acids (38.0%), arabinose (26.2%) and galactose (23.0%), while the citric acid soluble fraction GCA (3.1 g yield) presented uronic acids (23.9%) and arabinose (40.6%) as the major monosaccharides. The monosaccharide composition of both fractions indicated the presence of pectic polysaccharides that will be the focus of future investigation. These results were in agreement with the outcomes of earlier studies where pectins were extracted from other fruits that also belong to the Myrtaceae family, such as *Myrtus communis* L. (Myrtle) (Chidouh, Aouadi, & Heyraud, 2014) and *Psidium guajava* Linn (Guava) (Hua, Zhang, Huang, Yi, & Yan, 2014); these studies presented arabinose and galactose as the main neutral monosaccharides.

In contrast, the monosaccharide composition of fractions extracted with 2 mol L^{-1} and 4 mol L^{-1} NaOH indicated the presence of hemicelluloses. The fraction GA2M (7.9 g yield) showed mainly xylose (70.3%), suggesting the presence of a xylan, together with other hemicelluloses. When analyzed by ^{13}C-NMR, GA2M showed a spectrum with five main signals typical of (1→4)-linked D-xylans (data not shown). The signals of C1-C5, at δ 101.7, 72.7, 74.0, 75.6 and 63.2, respectively, could be assigned based on literature reports for *Mauritia flexuosa* (buriti) (Cordeiro, De Almeida, & Iacomini, 2015) and *Solanum lycopersicum* L. (tomatoes) (Nascimento, Baggio, Werner, Iacomini, & Cordeiro, 2016). Furthermore, evaluation of the fraction GA4M (1.4 g yield) presented glucose (37.5%), mannose (25.5%) and galactose (16.1%) as the major monosaccharides (Table 1).

Table 1. Monosaccharide composition of polysaccharides obtained from the pulp of gabiroba fruits.

Fraction	Monosaccharide composition (%)[a]						
	Rha	**Ara**	**Xyl**	**Man**	**Gal**	**Glc**	**UA[b]**
GW	3.4	26.2	1.9	2.7	23.0	4.8	38.0 ± 0.7
GCA	12.1	40.6	14.0	-	9.4	-	23.9 ± 0.9
GA2M	0.7	1.2	70.3	4.9	4.4	14.3	4.2 ± 0.3
GA4M	tr	1.0	13.5	25.5	16.1	37.5	6.4 ± 0.7
GA4MS	0.5	1.8	4.6	39.5	24.3	26.1	3.2 ± 0.5
GA4MP	-	2.3	21.1	27.7	19.3	29.6	-
GA4MS-FP	-	0.8	5.2	36.1	21.7	36.2	-

[a] % of peak area relative to the total peak area, determined by GC–MS. tr, traces.
[b] Uronic acids, determined using the *m*-hydroxybiphenyl method (Blumenkrantz & Asboe-Hansen, 1973).

In order to purify the main hemicellulose present in the fraction GA4M, a freeze-thawing treatment was carried out. The fractionation resulting from the freeze-thawing treatment is based on the differences in the branching degree of polysaccharides that confers different solubility. Those molecules presenting a structure closer to linear tend to precipitate, while those with a higher branching degree remain in the cold-water soluble fraction (Ruthes, Smiderle, & Iacomini, 2015). However, the branching pattern is not the only factor that could confer different solubility in cold water. The solubility of polysaccharides with different structures may be also due to monomer constitution, the linkage type and the molar mass (Whistler, 1973).

Thus, this first step of purification gave rise to a cold-water soluble fraction (GA4MS; 0.3 g yield) and a cold-water insoluble fraction (GA4MP; 0.9 g yield). Monosaccharide composition analysis revealed that GA4MP was comprised of mainly glucose (29.6%), mannose (27.7%), galactose (19.3%) and a higher amount of xylose (21.1%) in comparison to the GA4M (xylose 13.7%). The xylose present in this fraction is probably due the presence of xylans, as observed in the fraction GA2M, justifying its precipitation towards the freeze-thawing treatment, assuming that these xylans are less branched than the polysaccharides present on the cold-water soluble fraction (GA4MS). On the other hand, GA4MS presented small amounts of xylose (4.6%), uronic acids (3.2%), arabinose (1.8%) and rhamnose (0.5%) (Table 1), with mannose (39.5%), glucose (26.1%) and galactose (24.3%) as the main monosaccharides components.

GA4MS was treated with Fehling solution, yielding the fractions GA4M-FP (Fehling precipitate) and GA4M-FS (Fehling supernatant). Due to the low yield of the supernatant fraction (GA4MS-FS ~ 0.05 g yield) it was not analyzed further. Furthermore, the monosaccharide analysis of GA4MS-FP (0.2 g yield) revealed the presence of glucose (36.2%), mannose (36.1%) and galactose (21.7%) as the major monosaccharides, in a molar ratio of 1:1:0.6. However, small amounts of the xylose (5.2%), and arabinose (0.8%) were also observed, suggesting a possible contamination.

3.2 Structural analysis of fraction GA4M

The homogeneity of the fractions GA4M, GA4MS and GA4MS-FP was analyzed by HPSEC (Fig. 2A), detected by the RI. The elution profiles of GA4M and GA4MS showed peaks eluting at retention times between 50 and 60 min, in comparison to the fraction GA4MS-FP that showed a single elution peak at 52 min. This demonstrated that the

fractionation process adopted, using freeze-thawing and treatment with Fehling's solution, was efficient.

As the GA4MS-FP fraction was the only one that presented the homogeneous profile detected by the RI, the HPSEC chromatogram and the molar mass distribution of this fraction was performed (Figure 2B). Note that the light scattering chromatogram shows an invisible RI detected peak between 35 and 45 min, and the large fraction of molar mass is probably from an aggregate fraction. As there is no RI signal for the probable aggregated precursor, its (very low) concentration could not be estimated and, therefore, its corresponding molecular weight moments could not be calculated. However, for the successive peak at 52 min, clearly decreased in molar mass, was observed in light scattering and RI signals and molar mass distribution could be calculated as shown in Figure 2B.

The average molar mass (M_W) determined by HPSEC-MALLS-RI of GA4MS-FP was approximately 25,340 g mol^{-1}. This is in good agreement with results from the molar mass range of galactoglucomannans from angiosperms (Magnoliophyta), the kiwifruit GGM that present molar mass approximately between 16,900 and 41,800 g mol^{-1} (Schröder et al., 2001), and Artemisia GGM that showed M_W of 38,000 g mol^{-1} (Guo et al., 2012). These results are also in accordance to that found for GGM from gymnosperms as spruces (genus *Picea*) that have a molar mass range of 20,000 to 48,000 g mol^{-1}.

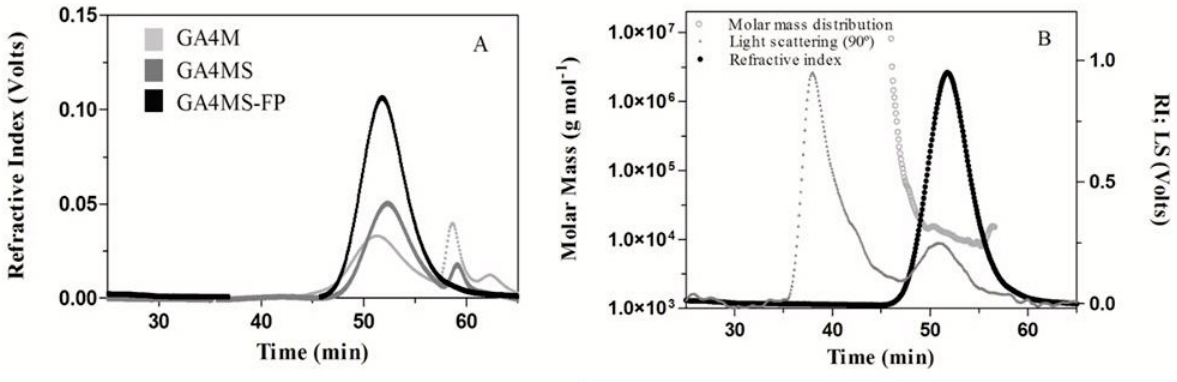


Fig. 2. **A**: HPSEC elution profile of fractions GA4M, GA4MS, and GA4MS-FP obtained from the pulp of gabiroba fruits. **B**: HPSEC elution profile of fraction GA4MS-FP detected by refractive index (RI), multi-angle laser light scattering (LS) and molar mass distribution.

To investigate the chemical structure of the hemicellulose present in the fraction GA4MS-FP, a methylation analysis was performed. The resulting per-*O*-methylated alditol acetates are shown in Table 2 and Figure S2. The results show that the chemical structure of

the hemicellulose in fraction GA4MS-FP is mainly composed of D-Glc*p* (32.04%) and D-Man*p* (22.31%), both (1→4)-linked, in addition to D-Man*p* (12.36%) and D-Glc*p* (2.48%) 4,6-di-*O*-substituted, and D-Gal*p* 2-*O*-substituted (6.36%) and as non-reducing end (15.33%) units. Small amounts of D-Glc*p* (1.39%) and D-Man*p* (1.66%), present as non-reducing end units, could also be observed (Table 2). The methylation results were in agreement with the monosaccharide composition determined by GC-MS (Table 1). Thus, a galactoglucomannan (GGM) with a main chain consisting of (1→4)-linked D-Glc*p* and (1→4)-linked D-Man*p* was confirmed as the major hemicellulose in the fraction GA4M-FP. The D-Gal*p* units appeared as backbone branches, mainly at O-6 position of the D-Man*p* units.

The structure of the GGM obtained from the pulp of gabiroba fruits was seen to be similar to those described for apple and kiwifruit GGMs, which show backbones consisting of β-D-Glc*p* and β-D-Man*p* (1→4)-linked and 6-*O*-substituted by D-Gal*p* units (Nara, Ito, Kato, & Kato, 2004; Schröder et al., 2001). However, differences were observed in the molar ratio of the monosaccharides. Gabiroba GGM presented Man:Glc:Gal in a molar ratio of 1:1:0.6, which is very similar to kiwi GGM that showed a molar ratio of 2:2:1 (Schröder et al., 2001), while the apple GGM present a molar ratio of 5.7:3.0:1.1, showing an amount of mannose twice as large as the amount of glucose (Nara, Ito, Kato, & Kato, 2004).

Extracellular GGMs isolated from suspension-cultured cells of *Rubus fruticosus* (blackberry) (Chambat, Cartier, & Joseleau, 1987) and *Nicotiana plumbaginifolia* (tobacco) (Sims, Craik, & Bacic, 1997) also present structures like the one obtained in this work, with Man:Glc:Gal in a molar ratio of 1.2:1.4:1.0 and 1.0:1.1:1.0, respectively. The structures of GGMs extracted from the primary cell wall were different from those extracted from the secondary cell wall of gymnosperms. The main differences were related to the molar proportions of D-glucose and D-mannose that in spruce varies between 1:3 and 1:4 (Glc:Man ratio), and to the content and distribution of acetyl groups. In spruce GGMs the mannose units in the backbone can be up to 65% acetylated at C-2 or C-3 (Willför et al., 2003).

The presence of a small percentage of the D-Xyl*p* (1→4)-linked and 2,4-di-*O*-substituted and L-Ara*f* units (1→5)-linked was also observed in the fraction GA4MS-FP obtained from the pulp of gabiroba fruits, suggesting the presence of an arabinoxylan contaminating the GGM.

Table 2. Linkage analysis of a galactoglucomanan (fraction GA4MS-FP) from the pulp of gabiroba (*Camponanesia xanthocarpa*) fruits.

Partially *O*-Methylated alditol acetate [a]	**Rt** [b]	**%Area of fragment** [c]	**Linkage type** [d]
2,3,4-Me_3-Xyl	11.93	0.59	Xyl*p*-(1→
2,3-Me_2-Ara	12.44	0.81	→5)-Ara*f*-(1→
2,3-Me_2-Xyl	13.04	4.36	→4)-Xyl*p*-(1→
2,3,4,6-Me_4-Man	13.41	1.66	Glc*p*-(1→
2,3,4,6-Me_4-Glc	13.46	1.39	Man*p*-(1→
2,3,4,6-Me_4-Gal	13.68	15.33	Gal*p*-(1→
3-Me-Xylp	13.99	0.29	→2,4)-Xyl*p*-(1→
2,3,6-Me_3-Man	14.44	22.31	→4)-Man*p*-(1→
2,3,6-Me_3-Glc	14.51	32.04	→4)-Glc*p*-(1→
3,4,6-Me_3-Gal	14.60	6.37	→2)-Gal*p*-(1→
2,3-Me_2-Man	15.59	12.36	→4,6)-Man*p*-(1→
2,3-Me_2-Glc	15.64	2.48	→4,6)-Glc*p*-(1→

[a] GC-MS analysis on a VF-5ms capillary column.

[b] Retention time (min).

[c] Percentage of peak area of *O*-methylalditol acetates relative to total area, determined by GC-MS and corrected by the monosaccharide composition.

[d] Based on derived O-methylalditol acetate.

Galactoglucomannans (GGMs) often contain trace amounts of pectic polysaccharides, xyloglucan and arabinoxylan. These small contaminants have also been reported for the GGMs from fruits of kiwi (Schröder et al., 2001) and apple (Nara, Ito, Kato, & Kato, 2004). In general, GGMs are very difficult to remove from the cell wall, and require relatively strong alkali conditions for their extraction. This polysaccharide can be strongly associated with other cell wall components such as xyloglucans, and also has the capacity to bind to cellulose microfibrils as reported by (Nara, Ito, Kato, & Kato, 2004) when analyzing the galactoglucomannan extracted from apple.

1 D and 2D NMR spectra were employed to provide detailed structural information about the gabiroba GGM. The signal assignments, as summarized in Table 3, were in agreement with published data (Guo et al., 2012; Hannuksela & Du Penhoat, 2004; Nascimento, Baggio, Werner, Iacomini, & Cordeiro, 2016; Schröder et al., 2001; Sims, Craik, & Bacic, 1997).

Table 3. ^{1}H and ^{13}C-NMR chemical shifts (δ, ppm) of the galactoglucomannan - GGM from the pulp of gabiroba fruits [b].

Monosaccharide unit		**1**	**2**	**3**	**4**	**5**	**6**	
							a	**b**
→2)-α-D-Gal*p*-(1→	^{1}H	5.19	3.96	4.10	4.02	3.56	3.76	
	^{13}C	98.8	77.5	70.1	69.3	75.3	61.2	
α-D-Gal*p*-(1→	^{1}H	5.02	3.82	3.77	4.02	3.93	3.89	3.73
	^{13}C	98.9	68.5	71.5	69.3	68.7	60.4	
→4)-β-D-Man*p*-(1→	^{1}H	4.75	3.98	3.76	3.69	3.77	3.99	3.84
	^{13}C	100.0	70.8	73.8	78.6	71.5	60.4	
→4,6)-β-D-Man*p*-(1→	^{1}H	4.76	3.98	4.10	3.69	-	4.00	3.93
	^{13}C	100.0	70.8	70.1	78.6	-	67.1	
→4)-β-D-Glc*p*-(1→	^{1}H	4.52	3.37	3.62	3.69	3.63	3.84	3.73
	^{13}C	102.4	73.0	74.8	78.6	71.0	60.4	
→4,6)-β-D-Glc*p*-(1→	^{1}H	4.57	3.63	3.57	3.69	3.63	4.00	3.93
	^{13}C	104.4	71.0	73.8	78.6	71.0	67.1	
→4)-β-D-Xyl*p*-(1→	^{1}H	4.48	3.37	3.62	3.82	4.12/3.41	-	-
	^{13}C	101.6	73.0	74.8	76.6	63.01	-	

[a] Values were determined in D_2O at 70 ºC.

[b] Measured relative to acetone at ^{1}H (δ 2.22) and ^{13}C (δ 30.2).

The ^{13}C-NMR spectrum of the gabiroba GGM, shown in Figure 3, showed a high complexity of signals. Six major signals could be observed in the anomeric region (roughly from 105 to 95 ppm), viz. δ 104.4, 102.4, 101.6, 99.9, 98.8 and 98.7. These could be assigned to the C-1 of the different monosaccharide units that compose the structure as follows: D-Glc*p* 4,6-di-*O*-substituted, D-Glc*p* (1→4)-linked, D-Xyl*p* (1→4)-linked, D-Man*p* (1→4)-linked and D-Man*p* 4,6-di-*O*-substituted, D-Gal*p* non-reducing end units and D-Gal*p* 2-*O*-substituted, respectively.

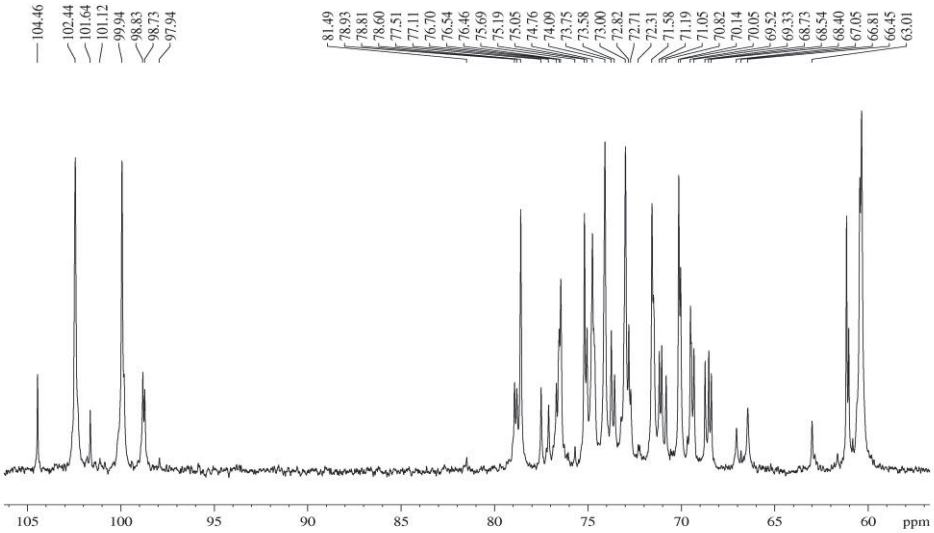


Fig. 3. ^{13}C NMR spectrum of the galactoglucomannan - GGM (fraction GA4MS-FP) from the pulp of gabiroba fruits, obtained at 70 °C in D_2O (chemical shifts are expressed in δ, ppm).

All signal assignments were confirmed by the analysis of ^{1}H-^{13}C heterocorrelated HSQC-DEPT NMR spectrum (Fig. 4). The presence of the characteristic signals of 4-*O*-substituted residues at δ 78.6/3.69 for both D-Glc*p* and D-Man*p* showed the glycosidic linkages of the gabiroba GGM. In addition, the O-6 substitutions for both D-Glc*p* and D-Man*p* units were confirmed from the negative phase signals (shown in red) (Fig. 4A; Table 3) at δ 67.1/4.00 and 3.93.

α- or β-configurations were determined by the coupling constants ($J_{C1/H1}$) of each signal in the anomeric region, found in $^{1}H/^{13}C$ coupled HSQC spectrum (data not shown). The high-field H-1 signals (Fig. 4B) indicated that D-Man*p* and D-Glc*p* units have a β-configuration; this was further confirmed by the coupling constants values of $J_{C\text{-}1/H\text{-}1}$ 158.0 and 163.1 Hz, respectively. Furthermore, the α-configuration, indicated by the low-field H-1 signals for the D-Gal*p* units, was confirmed by coupling constants values of $J_{C\text{-}1/H\text{-}1}$ 173.3 and 169.6 for the non-reducing end and 2-*O*-substituted units, respectively (Perlin & Casu, 1969). Based on the data from HSQC-DEPT NMR spectrum, the $^{1}H/^{13}C$ chemical shifts at δ 4.75/100.0, 3.98/70.8, 3.76/73.8, 3.82/76.6, 3.56/75.3, and 3.76/61.2 were assigned as H-1/C-1, H-2/C-2, H-3/C-3, H-4/C-4, H-5/C-5 and H-6/C-6 of (1→4)-linked β-D-Man*p* units respectively (Fig. 4A and B). The overlapping of the C-1 signal of β-D-Man*p* units 4, 6-di-*O*-

substituted was solved when analyzing the HSQC-DEPT spectrum (Fig. 4B). The H-1/C-1 signal of those units could be assigned at δ 4.76/100.0.

The $^{1}H/^{13}C$ chemical shifts at δ 4.52/102.4, 3.37/73.0, 3.62/74.8, 3.69/78.6, 3.63/71.0 and 3.84-3.73/67.1 were assigned as H-1/C-1, H-2/C-2, H-3/C-3, H-4/C-4, H-5/C-5, and H-6 (a-b)/C-6 of (1→4)-linked β-D-Glc*p* units respectively (Fig. 4A and B). The H-1/C-1 signal at δ 4.57/104.4 could be assigned to β-D-Glc*p* units 4,6-di-*O*-substituted (Fig. 4B).

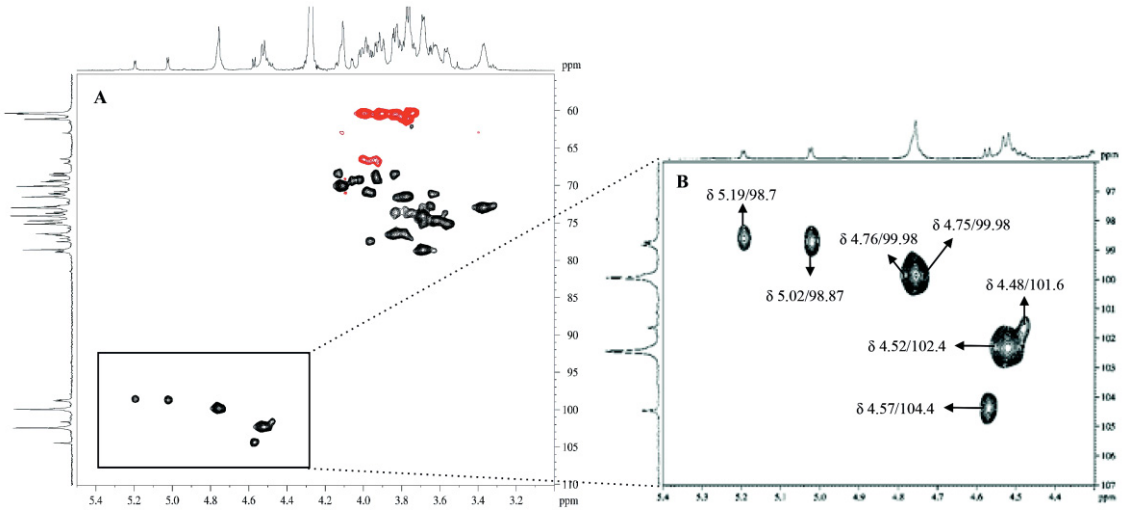


Fig. 4. $^{1}H/^{13}C$ HSQC-DEPT spectrum of the galactoglucomannan - GGM from the pulp of gabiroba fruits. (A) Total HSQC-DEPT spectrum; (B) Detailed anomeric region. The sample was dissolved in D_2O and data were collected at a probe temperature of 70 °C.

Regarding the side chains, the (1→2)-linked α-D-Gal*p* units showed a chemical shift at δ 5.19/98.8 in the anomeric region, while the non-reducing end α-D-Gal*p* units signal appeared at δ 5.02/98.9 (Fig. 4B; Table 3). Together with the linkage analysis data (Table 2), this suggests that these units were substituting the main chain at O-6 position mainly of the β-D-Man*p* units (12.36%) and to a lower extent of the β-D-Glc*p* units (2.48%).

The structural characterization of GGMs in *Malus domestica* (apple) and *Actinidia deliciosa* (kiwifruit) also showed the presence of single D-Gal*p* units and the disaccharide (1→2)-linked as side chains (Nara, Ito, Kato, & Kato, 2004; Schröder et al., 2001). Similar side chain structures were previously shown in GGMS isolated from *Artemisia sphaerocephala* Krasch (Guo et al., 2012) and *Nicotiana plumbaginifolia* (Sims, Craik, & Bacic, 1997). Based on the literature, the signals at δ 3.82/68.5 (H-2/C-2), δ 3.99/70.86 (H-3/C-3), δ 4.02/69.3 (H-4/C-4), δ 4.05/69.52 (H-5/C-5) and δ 3.73/60.4 (H-6/C-6) could be assigned to the T-Gal*p* units (Guo et al., 2012) (Fig. 4 A).

The interglycosidic correlations obtained through the analysis of the HMBC spectrum (Fig. 5) allowed the sequencing of the relevant long-range C-H connectivity of the constituent glycosyl residues. Accordingly, the H-1 of α-D-Gal*p* (1→2)-linked at δ 5.19, as well as the H-1 of the non-reducing end α-D-Gal*p* units at δ 5.02, was seen to be coupled to the ^{13}C resonance at δ 67.1 (C-6 of β-D-Man*p* 4,6-di-*O*-substituted). Other correlations were observed between: the H-1 of β-D-Man*p* (1→4)-linked and/or 4,6-di-*O*-substituted at δ 4.76, with the ^{13}C resonance at δ 78.6 [C-4 of β-D-Glc*p* (1→4)-linked and/or 4,6-di-*O*-substituted]; and the H-1 of β-D-Glc*p* (1→4)-linked and/or 4,6-di-*O*-substituted at δ 4.57 with the ^{13}C resonance at δ 77.5 [C-4 of β-D-Man*p* (1→4)-linked and/or 4,6-di-*O*-substituted] (Fig. 5A). The H-6 of β-D-Man*p* 4,6-di-*O*-substituted and of α-D-Gal*p* non-reducing end units at δ 3.99 and 3.89 were coupled with the ^{13}C resonance at δ 98.8 [C-1 of α-D-Gal*p* (1→2)-linked]; and the H-6 of β-D-Man*p* (1→4)-linked at δ 3.99 was coupled with the ^{13}C resonance at δ 104.4 (C-1 of β-D-Glc*p* 4,6-di-*O*-substituted). Taking into account the H-4 of β-D-Man*p* (1→4)-linked or 4,6-di-*O*-substituted at δ 3.69, a coupling with the ^{13}C resonance at δ 104.4 (C-1 of β-D-Glc*p* 4,6-di-*O*-substituted) could be observed. Another cross peak at δ 3.69 [H-4 of β-D-Glc*p* (1→4)-linked or 4,6-di-*O*-substituted], coupled to the ^{13}C resonance at δ 100.0 [C-1 β-D-Man*p* (1→4)-linked or 4,6-di-*O*-substituted], confirmed that both β-D-Man*p* and β-D-Glc*p* (1→4)-linked and 4,6-di-*O*-substituted were part of the main chain of the GGM obtained from the pulp of the gabiroba fruits (Fig. 5B).

These results confirmed the linkage analysis data and were in agreement with the data obtained for *Artemisia sphaerocephala* GGM (Guo et al., 2012). Here we could confirm that the α-D-Gal*p* units can substitute mainly the C-6 of the β-D-Man*p* units in the main chain, as single units or in a disaccharide unit (1→2)-linked. Based on the correlations obtained by HMBC, we could also confirm that both β-D-Glc*p* and β-D-Man*p* (1→4)-linked compose the main chain as suggested by the linkage analysis.

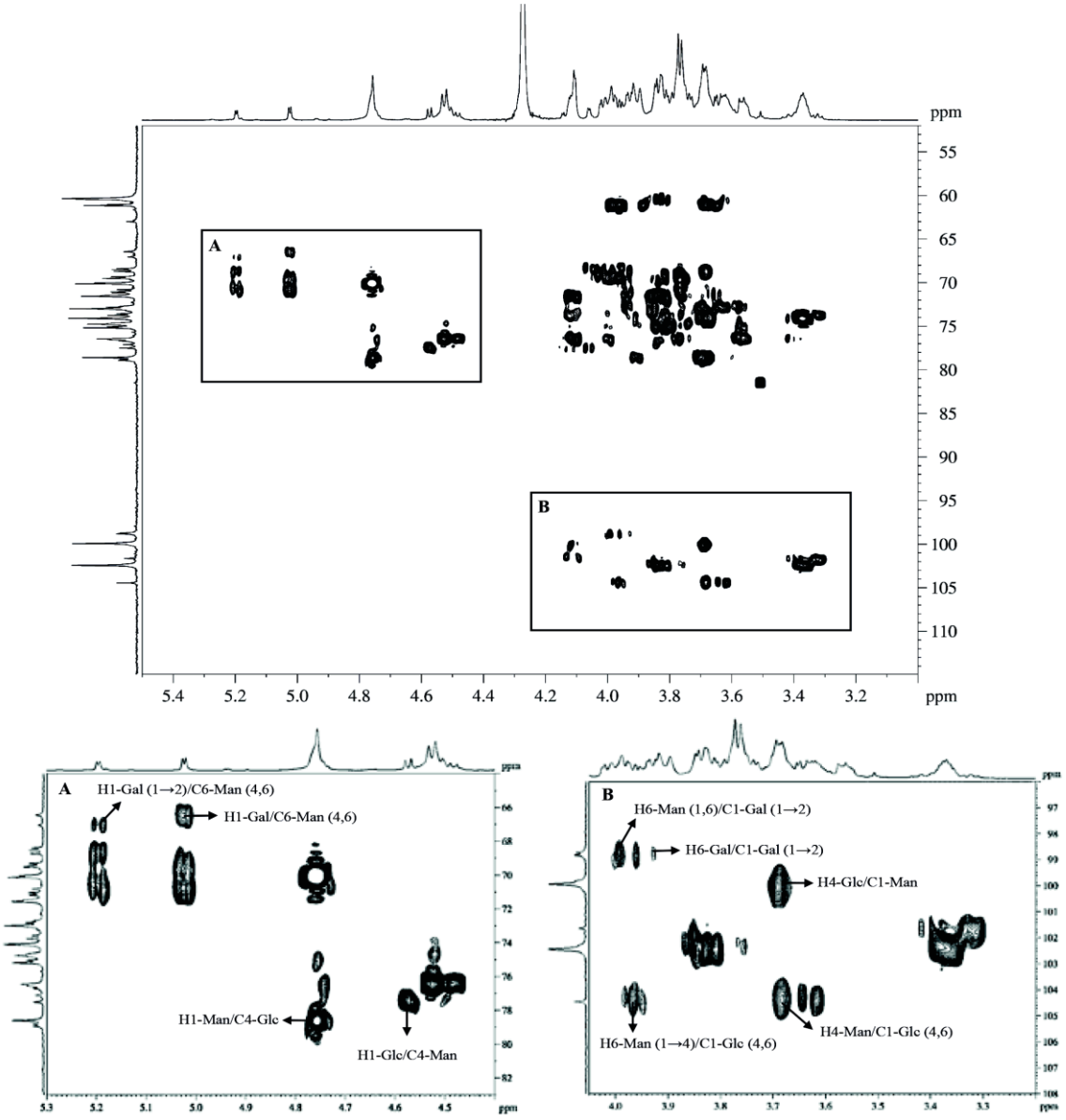
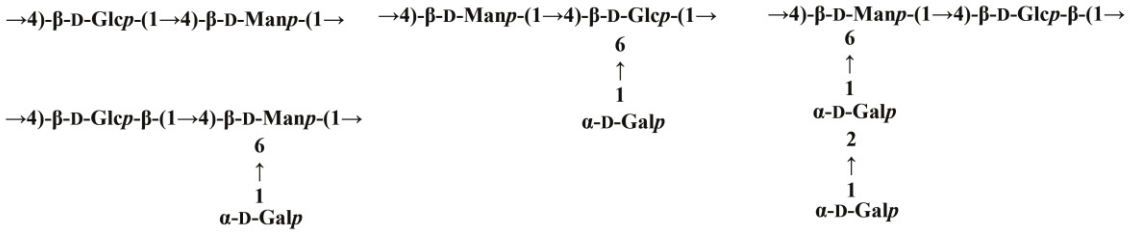


Fig. 5. HMBC spectrum of the galactoglucomannan - GGM (fraction GA4MS-FP) from the pulp of gabiroba fruits. Detailed HMBC spectrum of the (A) ^{1}H anomeric region, and (B) ^{13}C anomeric region; obtained at 70 °C in D_2O (chemical shifts are expressed in δ, ppm).

Based on all the results from analysis of the monosaccharide composition, methylation analysis, and 1D and 2D NMR spectroscopy, we propose that the following fragments compose the GGM characterized for GA4MS-FP fraction:



The gabiroba GGM (GA4MS-FP fraction) showed the characteristic structure found in GGMs extracted from the primary cell wall, as described for kiwifruit and apple GGMs (Nara, Ito, Kato, & Kato, 2004; Schröder et al., 2001). This structure was also similar to the extracellular GGMs isolated from suspension-cultured cells of blackberry (Chambat, Cartier, & Joseleau, 1987) and tobacco (Sims, Craik, & Bacic, 1997). However, the GGM structures already studied (Nara, Ito, Kato, & Kato, 2004; Sims, Craik, & Bacic, 1997; Xu et al., 2010) presented differences in the molar mass, in the degree of branching by D-Gal*p* side chains as well as in the distribution of D-Man*p* and D-Glc*p* units in the main chain. This makes the study of galactoglucomannans from different organisms important.

Besides the structural characterization of gabiroba GGM, the analyzes performed in the present study could explain and confirm the factors involved in the solubility differences of the fractionated polysaccharides (GA4M, GA4MS, GA4MP, GA4MS-FS and GA4MS-FP – Fig. 1). In the results, it was observed that the supernatant of the GA4MS fraction resulting from the freeze-thawing process, presented 8.2% more galactose units over the GA4M. The presence of D-Gal*p* units in the side chain provides more solubility of GA4MS in aqueous solution, reducing the intermolecular associations between the polymer chains. The presence of D-Gal*p* units as substituents on the gabiroba GGM (GA4MS-FP fraction) main chain was confirmed by ^{13} C-NMR analysis with the C1 signals at δ 98.8 and 98.9, which belong to D-Gal*p* 2-*O*-substituted and D-Gal*p* non-reducing ends, respectively. These ^{13}C NMR signals were confirmed by GC-MS methylation analysis, giving rise to (6.36%) of D-Gal*p* 2-*O*-substituted and (15.33%) of non-reducing end units.

According to Capek et al., 2000, for the galactoglucomannans isolated from spruce wood of *Picea abies* L. Karst, the presence of side chains with high amounts of D-Gal*p* units, greater than 5% increase the solubility in water. Else more, the presence of D-Gal*p* units in the side chain of galactomannans from the seeds of *Cyamopsis tetragonolobus* (WHISTLER, 1973) and *Mimosa scabrella* (Salvalaggio et al., 2015) inhibits the intermolecular associations of the mannose in main chain providing more solubility to the polysaccharides. These data

from GGM of gymnosperm (spruce wood) and from galactomannans of angiosperms (seeds) reinforced the hypothesis of the present study.

4 CONCLUSIONS

In the present study, a hemicellulosic fraction was obtained through alkaline extraction followed by purification of the pulp of the gabiroba fruits. This fraction (GA4MS-FP) was characterized to be mainly composed of a galactoglucomannan (GGM) with M_w of 25,340 g mol^{-1}. Analysis of the fine structure showed that the gabiroba GGM presented a backbone consisting of β-D-Glc*p* and β-D-Man*p* (1→4)-linked, 6-*O*-substituted by α-D-Gal*p* units. These results bring new insights into the structure of polysaccharides present in the primary cell walls of Myrtaceae members since, to our knowledge, there is no literature on the structural characterization of galactoglucomannans extracted from the pulp of fruits from Myrtaceae family.

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ARTIGO III

RHEOLOGICAL PROPERTIES OF PULP AND JAM OF GABIROBA (*Campomanesia xanthocarpa* Berg)

Este capítulo é uma reprodução do manuscrito intitulado "**Rheological properties of pulp and jam of gabiroba (*Campomanesia xanthocarpa* Berg)**" submetido em 26 de Outubro de 2017 no periódico Food Chemistry.

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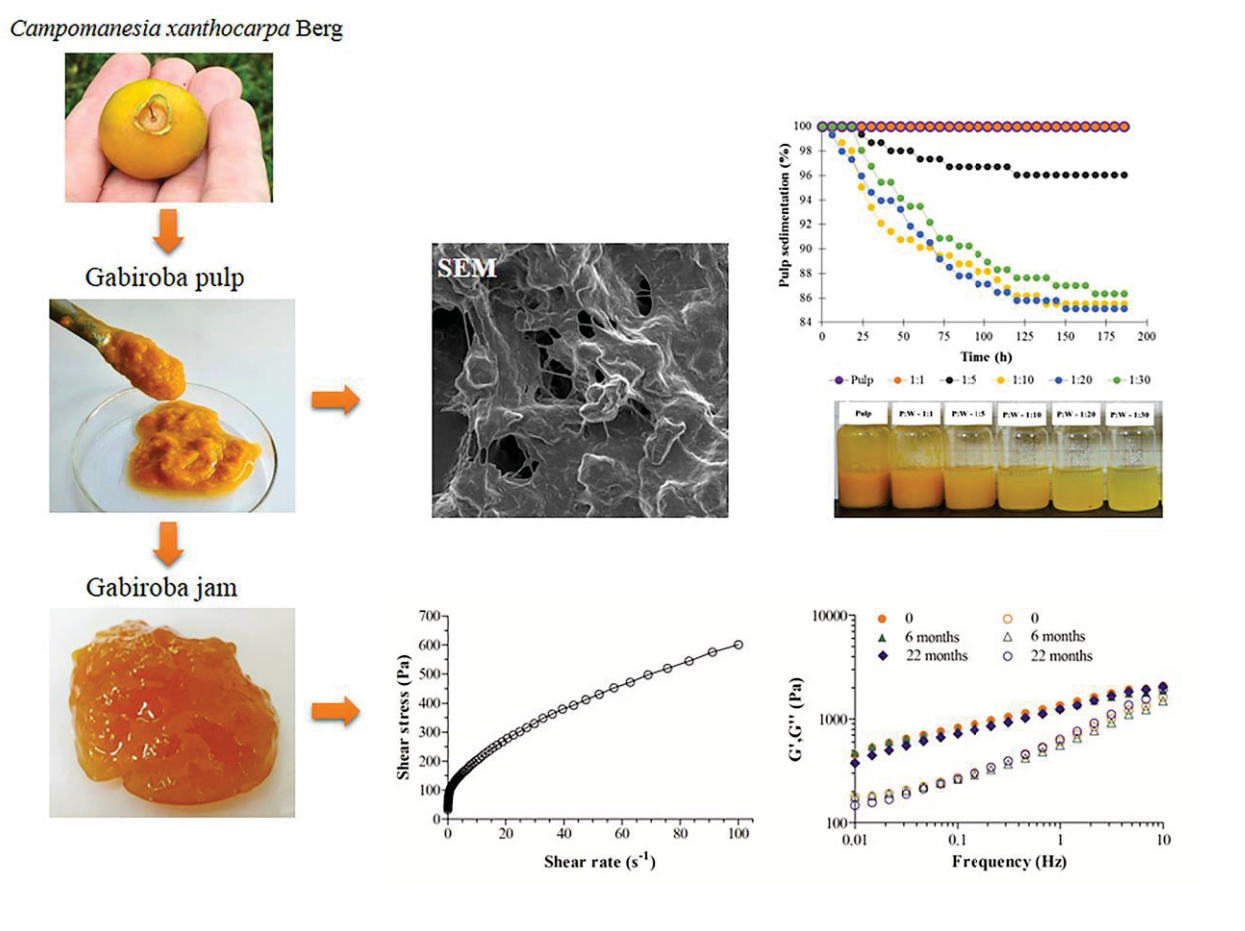
ABSTRACT

This study evaluated the rheological behavior of gabiroba pulp and its jam formulation. Gabiroba pulp presented a heterogeneous ultrastructure with a denser area formed by a compact mesh and a porous interface containing fibers. The fibers' presence promoted a slip effect when the gabiroba pulp was subjected to shear. Gabiroba pulp was characterized as a gel-like behavior with thermal stability. Gabiroba jam, developed using pulp as the raw material, behaved as a pseudoplastic fluid exhibiting yield stress described by the Herschel-Bulkley model. The dynamic oscillatory analysis showed that gabiroba jam typically behaved like a gel, i.e., G' values higher than the G'' in all frequency ranges evaluated. The results showed that gabiroba pulp is suitable for use as a raw material in the development of food products such as jam, encouraging the preservation of this native Brazilian species.

Keywords: Fruit pulp, Jam, SEM, Sedimentation analyses, Rheology

GRAPHICAL ABSTRACT



1 INTRODUCTION

The commercialization of native fruit plays an important role in social perspectives in countries around the world, including Brazil (Clerici & Carvalho-Silva, 2011). Brazil is the third-largest producer of fruit worldwide with about 45 million tons per year, of which 65% is consumed internally and 35% is destined for the foreign market. In 2016, approximately 31 thousand tons of fruit were exported worldwide specifically for food and canning products (Abrafrutas, 2017).

The utilization of local and seasonal fruits enhances the variety of fruits available on national and international markets and contributes towards sustainable agriculture. Recently, the demand for native and exotic products, particularly for food products, have increased, creating the necessity for a better understanding of the influence of processing on food structure and its properties (Ayala-Zavala et al., 2011).

Among the diversity of native fruit, numerous species from the Myrtaceae family (Barroso, 1978), 145 genera and 5,970 known species, are spread across the world (The plant list - Myrtaceae, 2013). One of them is the *Campomanesia xanthocarpa* Berg, popularly known as gabiroba, a Brazilian native species that is investigated in the present work.

Gabiroba fruit is round in shape, yellow or orange in color, has a transverse diameter of 2.5 cm and height of 2-3 cm, and has a thin, smooth epicarp. The endocarp is juicy with 2 to 6 seeds on average; the seeds are cylindrical, yellow, and have small black points that contain essential oil (Sanchotene, 1989; Lisbôa, Kinupp & Barros, 2011). The fruit is composed of 7% calyx, 16% seeds, 17% peel, and 60% pulp (Santos, Correia, Petkowicz & Cândido, 2012). Due to their nutritional properties, gabiroba fruits can be considered a functional food due to their high contents of water (79.1-83.5%), dietary fiber (4.1-9.8%), carbohydrates (7.8-10.2%), protein (1.0-1.1%), and lipids (0.7-1.9%) (Vallilo, Moreno, Oliveira, Lamardo & Garbelotti, 2008; Andrade, Helm, Mazza, & Mazza, 2012; Santos et al., 2012; Barbieri et al., 2017).

The nutritional properties of gabiroba pulp are associated with some other advantages, like high yield, attractive yellow color, and good stability, which make the pulp suitable to consume *in natura* and as a raw material with excellent properties for the beverage, ice cream, and jam industries (Lisbôa et al., 2011). It also has significant phenolic (19.59 μg g^{-1}) and 0.8% vitamin C (826.26 mg g^{-1}) contents. Recent Brazilian rankings ranked gabiroba as the third-richest fruit in vitamin C content (Valor nutricional da guabiroba, 2015).

Considering the demand for high-quality processed food, the rheological characterization of the viscoelastic properties of raw materials is important for the design, optimization, stability, and development of products with the highest final consumer acceptability (Fischer & Windhab, 2011; Augusto, Cristianini & Ibarz, 2012).

The rheological properties of fruit pulp belonging to the Myrtaceae family are still under-explored. Some species of the Myrtaceae family are reported in the literature as jabuticaba (*Myrciaria cauliflora*) (Sato & Cunha, 2007), araçá (*Psidium cattleianum* sabine) (Haminiuk, Sierakowski, Vidal & Massona, 2006), and goiaba (*Psidium guajava*) (De Oliveira, Rossi & De Barros, 2011), which have pulps that are characterized as having non-Newtonian behavior.

Among them, the gabiroba pulp (*Campomanesia xanthocarpa* Berg) showed the shear-thinning behavior described by the empirical rheological models of Ostwald Waale, Herschel-Bulkley, and the Power Law (De Oliveira et al., 2011; Santos et al., 2012). However, to date, no studies describe a more detailed rheological characterization of the gabiroba pulp.

In view of understanding the behavior of gabiroba pulp and its influence on a jam product, the aim of this work was evaluate the rheological properties of the pulp and identify the characteristics that influence the pulp as the raw material for the production of gabiroba jam. Since gabiroba jam is not produced commercially in Brazil, rheological measurements were employed to estimate the mechanical and thermal stabilities, which are essential parameters for the processing and storage of products, which influence the quality control and consumer acceptability of the developed product.

2 MATERIALS AND METHODS

2.1 Material

Ripe gabiroba (*Campomanesia xanthocarpa* Berg) fruits were collected from a conservation area in Irati-Paraná/Brazil, located at the geographic coordinates of 25° 25' south latitude, 50° 36' west longitude, and 25° 17' south latitude, 50° 30' west longitude. The fruits were selected and washed, the peel and seeds were removed in a Macanuda removing device (Model SPI-DMJI/2013), and the resulting pulp was frozen at -20°C for further analyses. A moisture analyzer (Model-OHAUS MB25) was used to evaluate the total solid content of the pulp. The chemical composition of the pulp was analyzed by energy dispersive spectrometry

analysis (EDS) by scanning electron microscopy (SEM) (VEGA3 LMU, Tescan, Kohoutovice, Czech Republic) at a 15kV accelerating voltage and equipped with a detector (SDD 80 mm^2) and AZ Tech Advanced software. The sample was put on aluminum stubs with double-face tape and coated with a thin layer of gold (SCD 030 – Pfeiffer, Balzers, Liechtenstein).

2.2 Jam cook-concentration process

Gabiroba jam was prepared using a fresh pulp and sucrose (purchased from the local market) (1:1 w/w), citric acid (P.A-ACS/Biotec) (0.2%), and commercial citrus pectin (GENU® - Rapid set/CPKelco) (0.2%). The required amounts of pulp and sucrose were mixed in an open pan, heated up to the boiling point, and concentrated by evaporation until the desired concentration of soluble solids content of 67.5º Brix, as measured with a digital refractometer (WYA/ABBE). The required amount of commercial pectin was separately dissolved in water with part of the sugar and allowed to hydrate under stirring for 24 h (Einhorn-Stoll, 2017). The pectin dispersion was added to the pulp-sugar mix towards the end of the concentration process. The citric acid was added to keep the pH around 3.5. To avoid pre-gelation, the citric acid solution was added just before the end of the process, and the pH was monitored with a digital pH meter (DMPH-1/Digimed). Finally, each sample was hot-filled at about 90 ºC into three sanitized and labelled glass jars, which were sealed with their screw tops and stored for later measurements.

2.3 Sedimentation analysis of gabiroba pulp

In the sedimentation test, the undiluted gabiroba pulp and five water-diluted samples, at pulp:water ratio (w/w) of 1:1, 1:5, 1:10, 1:20, and 1:30, were analyzed. The gabiroba pulp was firstly thawed and then diluted in ultrapure water - 18 Ω (Master system-MS2000/Gehaka) at the five proportions cited above. Each diluted sample was homogenized for 10 s in vortex. The sedimentation process, at 25 °C, was followed through photographs (without flash) for 186 h until stable sedimentation was reached, taking one photo every 30 min using a timer coupled to the camera (Canon 60 D with lens 24-105 mm F/4.0). The sedimentation analysis of the photographs was done through the ImageJ program, version 1.50 c (Java 1.7.0-45).

2.4 Rheological analyses

The rheological measurements were carried out using a Thermo Scientific Haake Mars II rheometer (Haake GmbH, Germany) equipped with parallel plate geometries: one smooth (20 mm diameter, 1 mm gap) for jam analysis, one smooth (35 mm diameter, 1.0 mm gap) for pulp analysis and another with a grooved surface (35 mm diameter, 1.0 mm gap) for freezing point analysis, respectively. The temperature was controlled by a circulating water bath (DC5-Haake K15), regulated by a Peltier (Haake UTM Controller), and kept at 25 °C (except for the temperature sweep analysis). The experiments were carried out in triplicate with mean and standard deviation evaluated by the statistical software *Graphpad Prism* 5. The samples were placed in the rheometer and kept for 5 min before analysis. A new sample was used for each repetition. The software used for the evaluation of data was HAAKE RheoWin Sofware, version 4.3.

2.4.1 Steady-state shear properties

The steady-state shear experiments for gabiroba pulp were carried out in the shear rate ($\dot{\gamma}$) range of 0.001-100 s^{-1} in CR mode (controlled shear rate) at 25 °C, using different annular gap sizes between the parallel plate geometry with gaps of 1.0, 1.5, 2.0, and 2.5 mm in parallel plate geometries with grooved surfaces. The steady-state shear experiments for gabiroba jam were carried out in the shear rate range of 0.01-100 s^{-1} for 300 s in CR mode at 25 °C with an annular gap size of 1.0 mm, in parallel plate geometries with smooth surfaces.

The flow behavior of the jam was fitted to the Herschel-Bulkley model. The Herschel-Bulkey model is represented by the equation $\tau = \tau_0 + K(\dot{\gamma})^n$, where τ is shear stress (Pa), τ_0 is yield stress (Pa), $\dot{\gamma}$ is shear rate (s^{-1}), K is the consistency coefficient ($Pa.s^n$), and n is the flow behavior index (dimensionless) that signifies the extent of deviation from Newtonian behavior (Rao, 2007). According to Steffe (1996), when the value of K is greater than 0 and the value of n is between $0 < n < 1$, the fluid is considered shear thinning.

2.4.2 Viscoelastic properties

Oscillatory stress sweeps between 0.01 and 100 Pa were performed for gabiroba pulp at a frequency of 1.0 Hz and at 25 °C to determine the linear viscoelastic range. Then,

frequency sweep measurements were carried out at 2.0 Pa, a shear stress value within the linear viscoelastic range; the frequency sweeps were from 0.01 to 100 Hz. The gabiroba jam was analyzed by frequency sweep (0.01-10 Hz) at 25 °C and at 1.0 Pa (determined by oscillatory stress sweep between 0.01 and 100 Pa, 1 Hz, at 25 °C).

2.4.3 Temperature sweep

The temperature dependence test was performed between 5 °C to 95 °C with a heating rate of 1 °C.min^{-1}, a constant frequency (1 Hz), and constant stress (2.0 Pa for pulp and 1.0 Pa for gabiroba jam). Next, a cooling step was performed using the same conditions as the heating experiment from 95 °C to 5 °C. The plate temperature was controlled by a Peltier effect device. Silicone oil was applied around the exposed sample surface and a solvent trap was used to permit efficient temperature regulation and prevent water evaporation.

The freezing point of the gabiroba pulp was assessed by decreasing the temperature from 25 °C to -10 °C and melting point temperature was assessed by increasing it from -10°C to 25 °C at a rate of 0.5 °C min^{-1}, constant frequency (1.0 Hz), and constant stress (2.0 Pa). The parallel plate geometry with grooved surfaces (35 mm diameter, 1.0 mm gap) was used.

3 RESULTS AND DISCUSSION

3.1 Steady-state shear properties of gabiroba pulp

In view of the nutritional properties of gabiroba fruits and their potential application in the development of food products, we evaluated the rheological behavior, structural morphology, and physical characteristics that can influence the use of gabiroba pulp as the raw material for developing products.

Figure 1 demonstrates the viscosity curve of gabiroba pulp and the dependence between the shear rate values and the annular gap size used. At 1 s^{-1}, the apparent viscosity decreases from 828 Pa.s to 157 Pa.s between the gap sizes of 1.0 and 2.5 mm, respectively. After the measurements were performed with different gaps, from 1.0 to 2.5 mm, the phase separation of insoluble particles within the analyzed pulp was clearly visible. The insoluble particles led to a slip (apparent wall slip) between the sample and the surface of the parallel

plate from the rheometer. The slip behavior of the gabiroba pulp sample may justify the differences in the viscosity analyses.

In the literature, the slip phenomenon and its influence on the apparent viscosity measurements was observed during the rheological measurements of particulate systems, such as foams, emulsions, suspensions, gels, and polymer solutions (Barnes, 1995; Bertola, Bertrand, Tabuteau, Bonn & Coussot, 2003). Tonon, Alexandre, Hubinger and Cunha (2009) also demonstrated the apparent wall slip in their studies with açai pulp (*Euterpe oleraceae* Mart.), relating the wall slip to the chemical composition of açai pulp (fibers, proteins, and lipids).

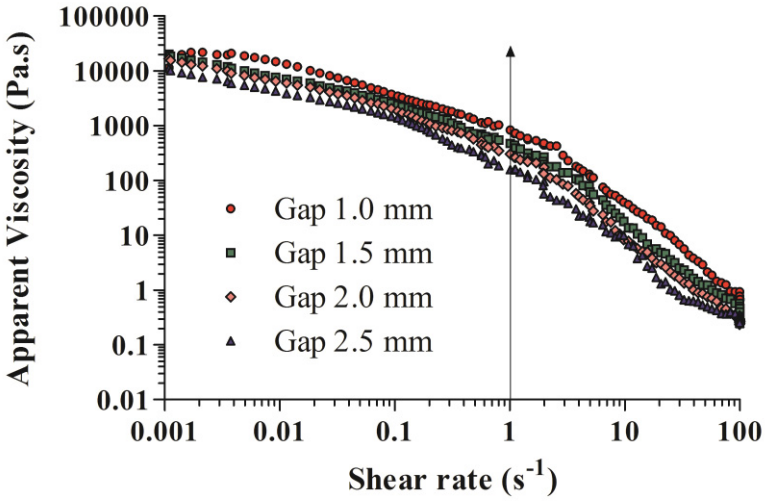


Fig. 1. Influence of shear rate on the viscosity curve of gabiroba pulp (0.001-100 s^{-1}) at 25 ºC, in parallel plate geometries with grooved surfaces using different gaps (1.0 to 2.5 mm).

Viscosity curves for gabiroba pulp were generated by evaluating shear rates from 0.001 to 100 s^{-1} using a geometry with grooved surfaces to avoid the formation of apparent wall slip. According to the literature, the serrations, when dipped into the bulk fluid, provide a good grip on the sample, eliminating or decreasing the occurrence of slip effects (Tonon et al., 2009); however, for the gabiroba pulp, the slip effects occurred for both the smooth (data not shown) and grooved geometries (Fig. 1). Therefore, in practice, as described by Moller, Mewis and Bonn (2006), a homogeneous flow does not occur for particulate systems when subjected to shearing because only a small region of the material actually moves, as demonstrated by gabiroba pulp on steady-state shear.

In the literature, the studies related to the steady-state shear properties of fruit pulps showed a shear thinning behavior that is mainly described by the Herschel-Bulkley model

(Tonon et al., 2009; Augusto et al., 2012). However, in those studies, the pulp was previously submitted to different processes to obtain a homogeneous pulp before performing the rheological measurements. For açai (*Euterpe oleraceae* Mart.) (Tonon et al., 2009) and siriguela (*Spondias purpurea* L.) (Augusto et al., 2012), the pulp was homogenized with a magnetic stirrer. On the other hand, the araçá (*Psidium cattleianum* sabine) pulp was put through a fruit depulper strainer with a screen of 1.5 mm sieves to get uniform particle size and consistency (Haminiuk et al., 2006). In the present study, the gabiroba pulp was not submitted to any type of processing.

3.2 Scanning electron microscopy (SEM) and energy-dispersive spectrometry analysis (EDS) of gabiroba pulp

The surface ultrastructure of gabiroba pulp was studied with the use of SEM (Fig. 2). We can observe in Figure 2, in the scanning electron micrographs, the panoramic images showing the mesh pattern, architecture and ultrastructure of the gabiroba pulp at the magnifications of 150 x (A), 300 x (B, C), and 700 x (D). In image (A), two different ultrastructure patterns can be observed. A dense mesh formed by juxtaposed aggregates present in the pulp of irregular-looking (▶), and in the central portion of this mesh, larger and smaller diameter perforations were observed, in which the fibers form a more open mesh (➡). Image (B) shows a more compact mesh, which presents a flatter, smooth, and regular face interposed with a structure forming aggregates, i.e., lumpy portions (⇨). Images (C) and (D), in greater magnitude, showed the lower-density area of this pulp, where it is possible to observe the perforations as well as the pattern of the fibers that make up this mesh (≫).

These images show that the gabiroba pulp presents a heterogeneous ultrastructure composed of two polymerization interfaces, a denser area formed by a compact mesh (soluble portion) and a porous interface containing fiber (insoluble portion). These characteristics support the steady-state rheological results that suggested that the apparent wall slip effect is related to the presence of the fibers that make up the pulp. This information can be further useful to optimize the technological properties of gabiroba pulp.

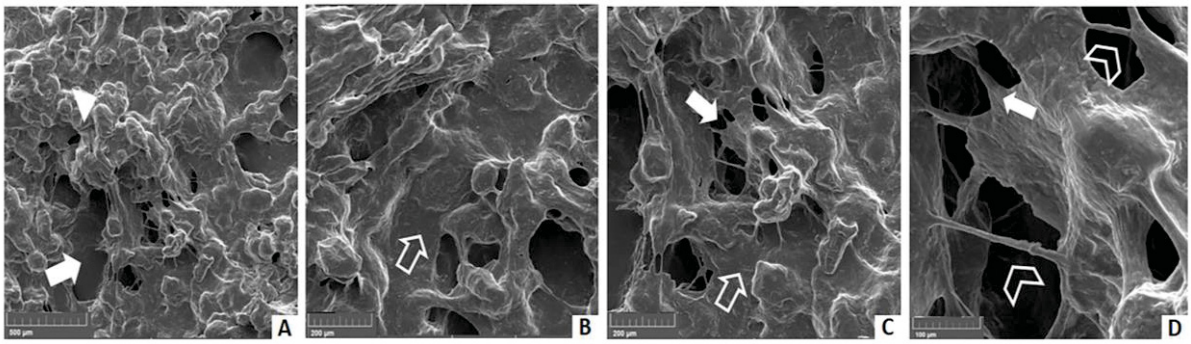


Fig. 2. Ultrastructural analysis in scanning electron microscopy (SEM) for the gabiroba pulp. The image (**A**) shows a panoramic image. The image (**B**) shows a greater magnification of the denser area. The images (**C**) and (**D**) show the area of lower density with characteristic perforations and the presence of fibers. Image magnifications: (A) 150 x, (B) 300 x, (C) 300 x, and (D) 700 x. This experiment was carried out in triplicate. The white arrows show: (▶) A dense mesh formed by juxtaposed aggregates of irregular appearance. (➥) Perforations of larger and smaller diameters and the presence of fibers, characteristic of a more open, less dense mesh. (⇨) The polymerization pattern of the compact mesh with a flatter, smoother, and regular face. (≫) The polymerization area of lower density with characteristic perforations and fibers.

The pulp of gabiroba fruit, after the removal of the peel and seeds, presented a total solid content of 17.7 % ± 0.8. Therefore, in order to determine the elemental chemical composition of gabiroba fresh pulp, the energy dispersive spectrometry analysis (EDS analysis) by SEM was performed. This analysis showed that gabiroba fruit mainly contained carbon (67%) and oxygen (30.8%), followed by potassium (1.8%), phosphorus (0.2%), sulfur (0.1%), magnesium (0.1%), and calcium (0.1%). The presence of carbon and oxygen as the major compounds is expected due to the amount of carbohydrates in the gabiroba pulp (7.8-10.2% as dry pulp) (Santos et al., 2012; Barbieri et al., 2017), notably pectins and hemicelluloses (galactoglucomannans), as reported recently (Barbieri et al., 2017).

3.3 Sedimentation analysis of gabiroba pulp

The gabiroba pulp sedimentation process was evaluated for undiluted pulp and for five pulp:water ratios (1:1, 1:5, 1:10, 1:20, and 1:30, w/w) as shown in Figures 3A and 3B.

Undiluted pulp and pulp-water (1:1 ratio) samples remained stable with a single visible phase; pulp precipitation was not observed for any of the samples during the sedimentation period evaluated (Fig. 3B). The stability of these two samples is due probably to the fiber content, as shown by the porous interface containing fibers (insoluble portion) in the surface ultrastructure of gabiroba pulp obtained by SEM analysis (Fig. 2). The literature

describes a fiber content of 4.1-9.8% for gabiroba pulp (Andrade et al., 2012; Santos et al., 2012; Valor nutricional da gabiroba, 2015). Fibers have swelling capacity and can increase water retention, preventing syneresis (Elleuch, Bedigian, Roiseux, Besbes, Blecker & Attia, 2011).

The sedimentation stability of undiluted pulp and the 1:1 pulp:water concentration suggest that gabiroba pulp has potential for use in the preparation of concentrated juices without the presence of additives. This feature is interesting since most fruit juices and nectars use some kind of additive in their formulation to increase viscosity, stabilize them, and prevent pulp sedimentation (Coelho et al., 2017).

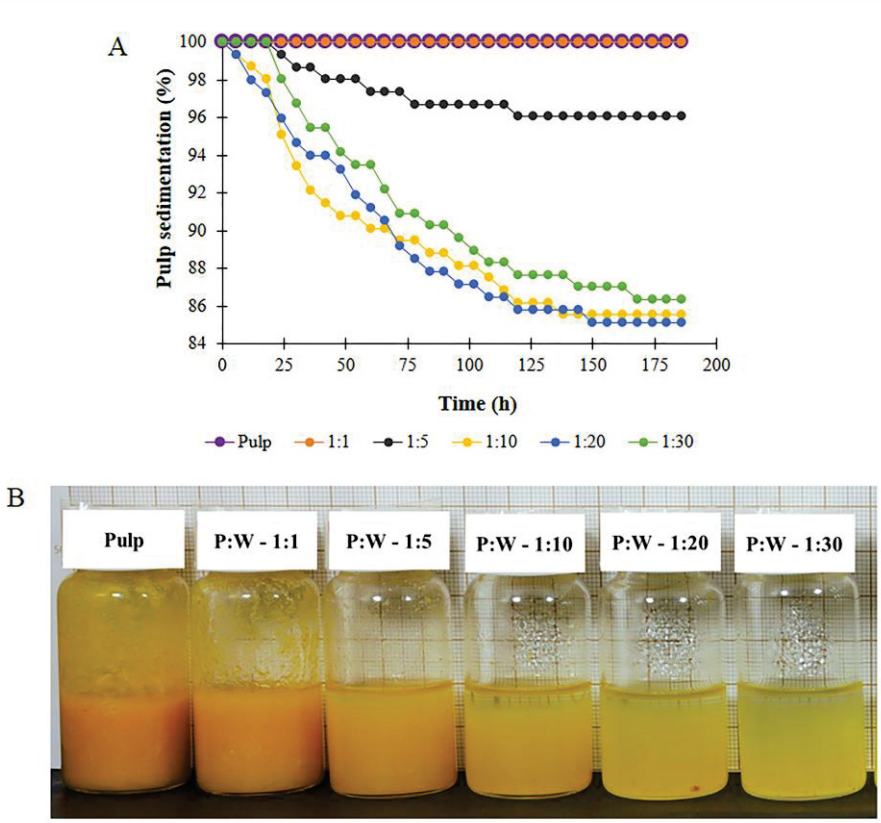


Fig. 3. Sedimentation analysis of gabiroba pulp during 186 hours. **(A)** Sedimentation of gabiroba pulp as a function of time. **(B)** Undiluted pulp (**Pulp**) and pulp:water ratios **(P:W)** from 1:1 to 1:30, after sedimentation stability.

After 186 h of sedimentation, the other dilutions analyzed had sedimentation rates of 4%, 15%, and 13% for the 1:5, 1:20, and 1:30 pulp: water ratios, respectively. Interestingly, even for the gabiroba pulp at the 1:30 pulp: water ratio, the sedimentation rate was only 13%; this result may suggest the use of gabiroba pulp for nectar formulations. Coelho et al. (2017), in their studies of passion fruit (*Passiflora edulis*) nectar, showed similar results, i.e.,

sedimentation rates between 10% and 15%; however, the authors proposed in the formulation of passion fruit nectar the addition of polysaccharides (carrageenan or guar) as thickening additives.

3.4 Dynamic rheology properties of gabiroba pulp

The dynamic viscoelastic properties of gabiroba pulp were evaluated by elastic (G') and viscous (G") moduli as a function of frequency (Fig 4A). Both moduli had a slight frequency dependence, with G' exceeding G" at all the frequencies analyzed (0.01-100 Hz). G' increased from 3,300 Pa to 13,500 Pa and for G'' from 900 Pa to 6,000 Pa at 0.01 and 100 Hz, respectively. The values of G' higher than G" characterize the gabiroba pulp as a material with gel-like behavior. This behavior, obtained by a dynamic oscilatory system, can be attributed to the chemical structure of gabiroba pulp, more specifically the 6.5-8.5% pectin content (Santos et al., 2012; Barbieri et al., 2017) and other polysaccharides present in the gabiroba pulp. This characteristic of gel-like behavior also may explains the stability of gabiroba pulp during the sedimentation process (Fig 3A).

A gel-like behavior has been reported for other pulp fruits, such as jabuticaba (Sato & Cunha, 2009), siriguela (Augusto et al., 2012), and açai pulp (Tonon et al., 2009). However, the açai pulp analyzed by Tonon et al. (2009) in the frequency of 1 Hz at 25 ºC presented G' and G'' around 150 Pa and 30 Pa, respectively, which are smaller values than the values of the modules (G' 7,000 Pa; G'' 2,000 Pa) obtained for the gabiroba pulp under the same conditions.

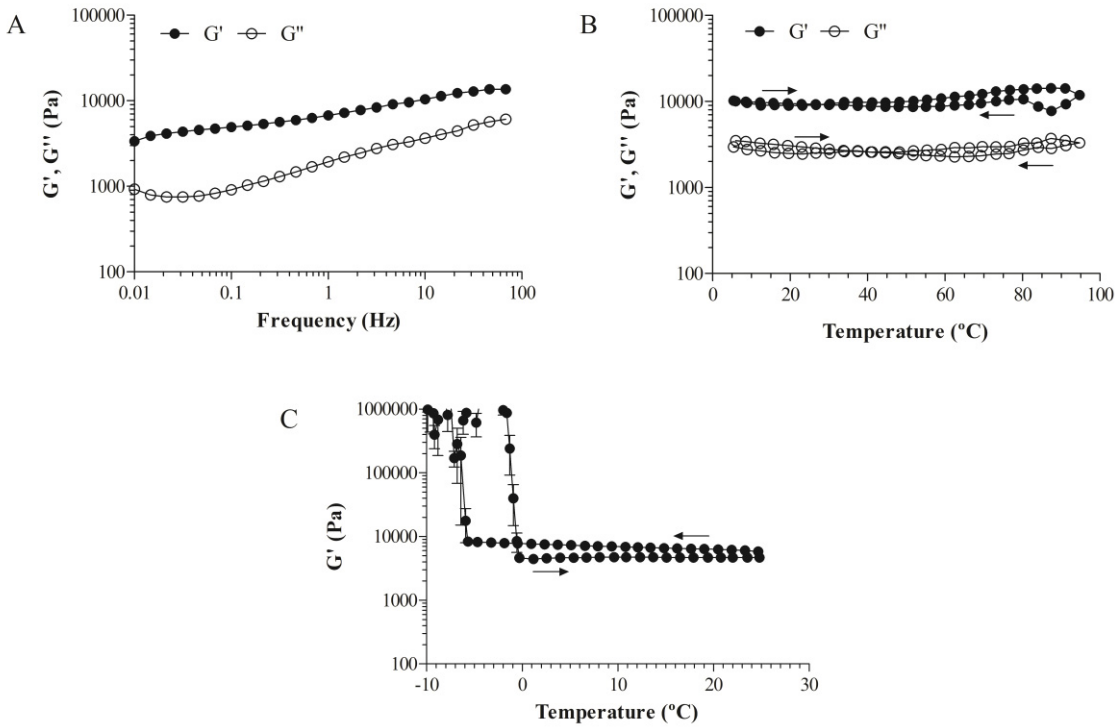


Fig. 4. Dynamic rheology properties of gabiroba pulp. (**A**) Frequency sweep (0.01 – 100 Hz) at 25 ºC, showing the frequency dependence of the elastic modulus (G', full symbols) and viscous modulus (G'', open symbols). (**B**) Elastic modulus (G') and viscous modulus (G'') as a function of temperature for heating and cooling cycles (5-95ºC) with a constant temperature rate of 1 °C.min^{-1}, at 1.0 Hz and 2.0 Pa, using the geometry with a smooth surface. (**C**) Freezing point dependence of the elastic modules (G'). Temperature sweep at the 25 ºC to -10 ºC range (cooling and heating cycles) at 1.0 Hz, 2.0 Pa, at a constant temperature rate of 0.5 °C.min^{-1} using the geometry with a grooved surface.

In order to investigate the thermal stability of the gabiroba pulp, temperature sweeps at a constant frequency of 1 Hz were performed. Figure 4B shows G' around 10,000 Pa higher than G" around 3,500 Pa, with the moduli values stable throughout the heating-cooling cycle (5 °C - 95 °C). The data obtained for gabiroba pulp characterize the predominance of elastic behavior, like a gel, and showed the thermal stability of the pulp at a wide range of temperatures, reflecting low internal structure changes in the temperature range analyzed. The siriguela pulp also presented thermal stability, but in a smaller temperature range (0 °C – 40 ºC) (Augusto et al., 2012). The thermal stability of gabiroba pulp indicates that the pulp can be used for different applications involving heating, such as jam and fruit filling in bakery products. In addition, it shows that the gabiroba pulp is suitable for pasteurization, which is generally done from 60 ºC to 100 ºC to reduce the growth of microorganisms, as well

as extending shelf life and delaying deterioration in industrialized fruit pulp (Saeeduddin et al., 2015).

The stability of gabiroba pulp was also evaluated using a freezing test (Fig. 4C). The freezing test is a way to simulate the conditions that may be used by the consumer to store the pulp. In this test, G' was determined throughout the heating–cooling cycle between 25 ºC and -10 ºC, at a constant cooling rate of 0.5 °C.min^{-1} and constant frequency of 1 Hz, using the geometry with a grooved surface.

Fig 4C shows, upon cooling, a constant value of G' around 7,000 Pa from 25 ºC until -10 ºC. Suddenly, a large increase of G' (800,000 Pa) was observed at -6 °C. The abrupt increase of G' demonstrates the liquid-solid phase transition of the water particles present in the pulp, by which it was possible to determine the freezing point of gabiroba pulp.

Upon heating, a large decrease of G' (from 800,000 to 5,000 Pa) was observed on a smaller range of temperatures (from -2 ºC until 0.3 °C), compared to that of the cooling cycle. After that, G’ remained constant (around 5,000 Pa) until the end of the temperature sweep (0 °C - 25 ºC). Meanwhile, the mechanical spectrum recorded after 0 °C at both cycles, heating/cooling, demonstrated the constant values of G’ throughout the temperature sweep (0 °C - 25 ºC). This behavior during the freezing test demonstrates that the macromolecular characteristics of the gabiroba pulp remain stable even after freezing and thawing, suggesting that the freezing process can be used as a process of storage and preservation of gabiroba pulp without changes in the pulp’s rheological properties. In addition, the results obtained for gabiroba pulp by the temperature sweep during the freezing and thawing tests through rheological analysis showed that the heating-cooling cycle can be used as a way to monitor the effects that the freezing process causes in the viscoelastic behavior of the fruit pulp. The results can also help to monitor the consistency and texture that must be maintained for the safety and nutrition quality of frozen products.

3.4.1 Steady-state shear properties of gabiroba jam

The gabiroba pulp was characterized as having a gel-like behavior (Fig 4A) and thermal stability in a wide range of temperatures (Fig 4B), demonstrating that this pulp has favorable rheological and physico-chemical properties for its use as raw material in food products. Therefore, a gabiroba jam formulation was developed and the rheological properties of the jam were investigated.

The flow behavior of gabiroba jam was evaluated at 25 ºC (Fig. 5A). The shear stress rose with increases in the shear rate and the sample exhibited a yield stress minimum of approximately 108 Pa to initiate the flow. The yield stress value obtained was similar to that found for mango jam, 112 Pa (Basu & Shivhare, 2010). The existence of yield stress in the flow of a material indicates that there is a cross-linked structure or other interactive structure that must be broken down before flow can occur at an appropriate rate. In this type of fluid, the shear stress curve does not begin at the origin of the shear stress/shear rate plot and is concaved downwards (Canet, Alvarez, Fernández & Luna, 2005; Sun & Gunasekaran, 2009). The presence of a yield stress is a typical characteristic of multiphase materials, such as fruit pulps and juices, which are formed by a dispersion of insoluble components (materials of cellular walls) in a water solution (serum, containing carbohydrates, minerals, proteins, and soluble polysaccharides) (Sun & Gunasekaran, 2009).

The flow behavior was fitted to the Herschel-Bulkley model with an obtained R^2 value of 0.987, similar to mango jam (Basu & Shivhare, 2010), with R^2 values ranging between 0.818 and 0.996, and apple jam (Tan, Cui, Lu, Zhao & Wang, 2014), with R^2 values ranging from 0.891 to 0.998.

By the Herschel-Bulkley model, the parameters, such as τ_0 (yield stress), K (consistency coefficient), and n (flow behavior index), may be determined, indicating the extent of deviation from Newtonian behavior (Steffe 1996; Rao, 2007). The yield stress obtained for gabiroba jam was (τ_0) 32.27 Pa. The K value of 39.40 Pa.s^n and the n value of 0.57 ($n<1$) indicate that gabiroba has a shear thinning behavior. In relation to K, the higher its value, the more consistent (or viscous) is the jam (Steffe, 1996; Rao, 2007); the K value found for gabiroba jam (39.40 Pa.s^n) at 25 ºC was greater than that described for rose hip marmalade (17.6 Pa.s^n) at 25 ºC (Sagdic, Toker, Polat, Arici & Yilmaz, 2015) and within the range (21.0 to 73 Pa.s^n) shown by Falguera, Mengual, Vicente and Ibarz (2010) for peach jam at 20 ºC, for which the K value varied according to the ingredients of their formulations.

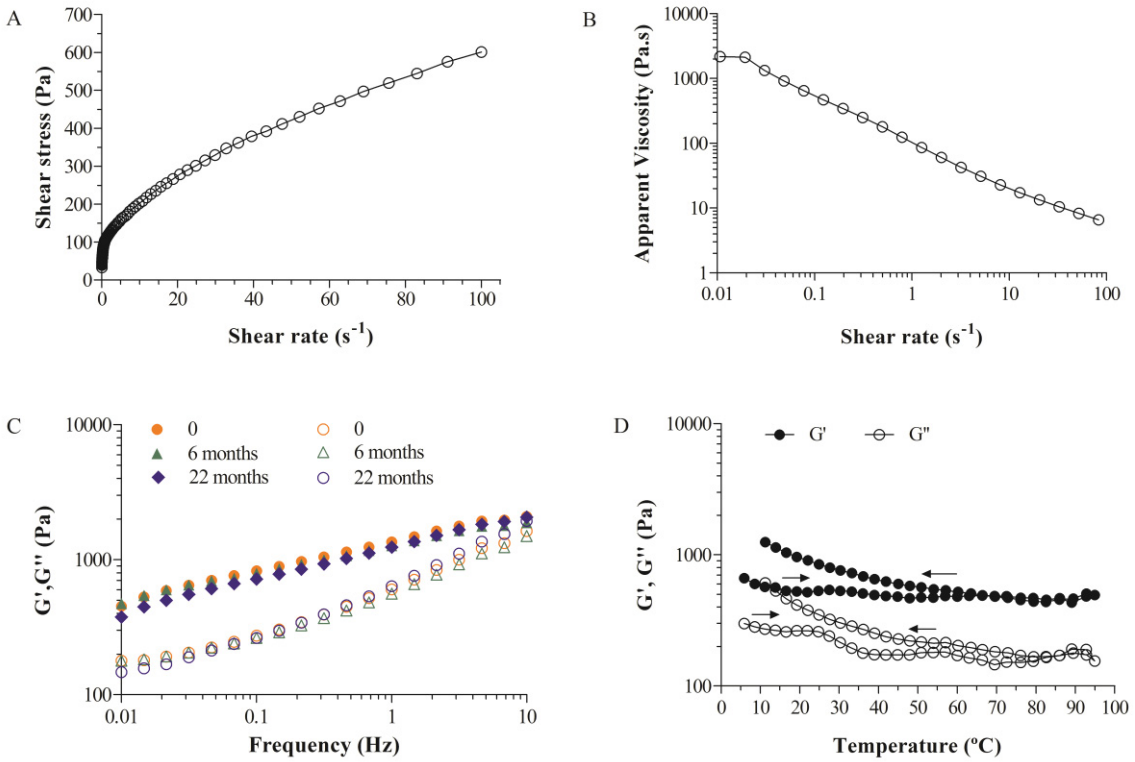


Fig. 5. Influence of shear rate on the flow curve (**A**) and viscosity curve (**B**) of gabiroba jam (0.01 – 100 s^{-1}) at 25 ºC. (**C**) Frequency sweep (0.01 – 10 Hz) at 25 ºC, showing the effect of storage time on rheological behavior of gabiroba jam. Elastic modulus (G', full symbols) and viscous modulus (G'', open symbols) (**D**) Elastic moduli (G', full symbols) and viscous moduli (G'', open symbols) as a function of temperature for heating and cooling cycles (5-95 ºC) of gabiroba jam, with a constant temperature rate of 1 °C.min^{-1} at 1.0 Hz and 1.0 Pa.

Figure 5B shows the gabiroba jam viscosity as a function of shear rate. The shear thinning behavior was observed as the apparent viscosity decreased and the shear rate increased. The viscosity curve showed a Newtonian plateau at a low shear rate (0.01 - 0.02 s^{-1}) with an apparent viscosity of 2,000 Pa. When analyzed at 50 s^{-1}, the apparent viscosity was 8.3 Pa.s, higher than the apparent viscosity of the peach jam (3.5 Pa.s) (Falguera et al., 2010). The shear thinning behavior was also observed in other fruits jams, such as mango (Basu & Shivhare, 2010) and apple jam (Tan et al., 2014), as determined by the flow curve.

3.4.2 Dynamic rheology properties of gabiroba jam

The determination of jams' viscoelastic characteristics is important for suitable design and handling processes; therefore the dynamic viscoelastic properties of gabiroba jam

were evaluated by frequency sweep (Fig. 5C). Elastic (G') and viscous (G") moduli increased with increasing frequency and both demonstrated a dependency on frequency. For gabiroba jam, the G’ values were higher than the G’’, which is the typical behavior of gels with a predominantly elastic character.

The effect of storage time on the rheological properties of gabiroba jam was also analyzed by frequency sweep at 25 °C. At the initial time, just after formulation (time 0), G 'and G” values were approximately 1,300 and 600 Pa, respectively, at frequency of 1.0 Hz. The values of both moduli remained very similar to the jam at time 0, even after storing the jam for 6 and 22 months (Fig. 5C), demonstrating the maintenance of its rheological properties. The pomegranate jelly showed stable rheological characteristics for 8 weeks in relation to the effect of storage time (Ventura et al., 2013). These results obtained for the gabiroba jam suggest that the product could be stored for a long period while maintaining its rheological characteristics, which is important for jam formulations that must maintain the gel texture and consistency.

As a food product goes through temperature variations from its manufacture to storage and commercialization, the rheological properties of gabiroba jam were evaluated as a function of temperature for heating and cooling cycles (5-95ºC) at a constant frequency of 1 Hz (Fig. 5D). During the heating cycle (from 5 ºC to 95 °C), a decrease of G’ (from 664 Pa to 494 Pa) and G’’ (from 298Pa to 155 Pa) moduli was observed. The decrease of both moduli during the heating cycle can be related to the changes of polymer chains’ flexibility, as well as the rupture of junction zones of network pectin and other polysaccharides. However, during the cooling cycle (from 95 ºC to 5 °C), higher values of G' (1,253 Pa) and G" (610 Pa) moduli were observed, compared to both moduli values at 5 °C during the heating cycle (G' 664 Pa; G" 298 Pa), demonstrating a temperature dependence of G’ and G’’ throughout the heating-cooling cycle. The increase of G' during the cooling of the jam can be explained by the strengthening of the gel structure due to the gelation process of the pectin along with the temperature decreases. The same rheological behavior as a function of temperature was also observed by Garrido, Lozano and Genovese (2015) in the cooling of apple jelly (90 ºC to 20 ºC) and by Genovese, Ye and Singh (2010) in a mixture of high methoxyl (HM) pectin and apple particles.

The gelation mechanism of HM pectin is given by the non-covalent attachment of adjacent pectin chains, leading to an interconnected three-dimensional network, which is stabilized by hydrogen bonds and hydrophobic interactions between the methyl ester groups of the pectin chains (Kastner, Kern, Wilde, Berthold, Einhorn-Stoll & Drush, 2014). In

addition, since gabiroba pulp exhibits elastic gel-like behavior and thermal stability (Fig. 4B), the concentrations of gabiroba pulp particles may also influence the rigidity of the three-dimensional network formed by the HM pectin present in the jam. This effect may be because fruit particles replace water in the final composition of the composite gels (Genovese et al., 2010). The decrease of water activity favors the hydrophobic interactions between pectin chains, increasing the strength of the gel network.

4 CONCLUSIONS

This study showed, through ultrastructure, sedimentation and rheological analyses, the potential of gabiroba pulp for consumption *in natura* or for use as raw material in the development of food products. Gabiroba pulp analyzed by SEM presented a heterogeneous ultrastructure with a denser area formed by a compact mesh and a porous interface containing fibers. The fibers' presence promoted a slip effect when the gabiroba pulp was subjected to shear; however, when the pulp, both undiluted and diluted in water, was analyzed through the sedimentation process, it was observed that the swelling capacity of fibers increased water retention, preventing the sedimentation of the pulp.

The dynamic viscoelastic properties of gabiroba pulp showed that the pulp presents a gel-like behavior and it is thermally stable in the temperature range of 5-95 ºC. The mechanical spectrum recorded after heating/cooling cycles (25 to -10ºC) showed that the macromolecular characteristics of the gabiroba pulp remain stable even after the process of freezing and thawing. Thus, it was demonstrated that the gabiroba pulp can be preserved and stored by freezing and that, even after thawing, the viscoelastic properties of the pulp are maintained.

The flow behavior of a gabiroba jam, developed using pulp as raw material, was analyzed and the jam exhibited a yield stress minimum to initiate the flow. The pulp had a shear thinning behavior described by the Herschel-Bulkley model. The mechanical spectrum and thermal stability analysis showed that the gabiroba jam presented the typical behavior of gels and could be stored until at least 22 months (valued period) while maintaining its rheological characteristics. The obtained results indicate that gabiroba jam may be a good way to use the gabiroba pulp. Also, the production of a jam could be an additional source of income for rural farmers in the regions where gabiroba is found, encouraging the preservation of this native Brazilian species.

CONFLICT OF INTEREST STATEMENT

The authors declare that they have no conflict of interest.

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3 CONCLUSÕES E CONTRIBUIÇÕES

Este trabalho foi desenvolvido abordando diferentes aspectos: 1) A caracterização da estrutura química das pectinas presentes na polpa de gabiroba e suas propriedades reológicas em solução; 2) A caracterização da estrutura química de hemiceluloses presentes na polpa de gabiroba; 3) O estudo do comportamento reológico da polpa de gabiroba (*in natura*) e de uma formulação de geleia produzida a partir da polpa.

Deste modo, de acordo com os resultados obtidos foi possível concluir que:

• Através da extração aquosa a quente foi possível extrair as pectinas da polpa de gabiroba, sendo que a fração bruta (GW) apresentou-se composta por homogalacturonanas e ramnogalacturonanas do tipo I. As análises também sugerem a presença de arabinogalactanas e/ou arabinanas e galactanas nesta fração, a qual ainda deve ser investigada através de análises de RMN bidimensional e metilação para comprovar suas estruturas e ligação na cadeia principal.

• A partir do fracionamento das pectinas presentes na fração GW por congelamento e degelo e tratamento com solução de Fehling, foi possível isolar a fração GWP-TEP, a qual apresentou-se principalmente composta por homogalacturonanas.

• Em relação ao comportamento de fluxo das pectinas (fração GW), as soluções em diferentes concentrações (1%, 3% e 5%) na presença de NaCl 0.1 mol L^{-1}, apresentaram comportamento pseudopástico. Além disso, foi observado um aumento na viscosidade de acordo com o aumento da concentração de pectina nas soluções.

• Nas análises reológicas oscilatórias, a solução de pectina na concentração de 1% apresentou comportamento viscoelástico com características de solução diluída. Já a concentração de 3% apresentou comportamento típico de solução concentrada, caracterizada pelo cruzamento dos módulos G' e G'' após a frequência de 1 Hz, enquanto que a solução na concentração de 5% apresentou comportamento típico de gel fraco. Foi observado que os valores dos módulos G' e G'' também aumentaram em resposta ao aumento da concentração de pectinas nas soluções.

• Através da extração com hidróxido de sódio na concentração de 4 mol L^{-1} foi possível extrair da polpa de gabiroba uma galactoglucomanana com cadeia principal formada por unidades de β-D-Glc*p* e β-D-Man*p* (1→4) ligadas e unidades de α-D-Gal*p* substituindo principalmente as unidades de β-D-Man*p* na posição O-6.

• As análises de microscopia eletrônica de varredura (SEM), mostraram que a polpa de gabiroba apresenta uma ultraestrutura heterogênea na qual foi possível observar a presença das fibras que compõem a polpa.

• O efeito de deslizamento observado nas análises reológicas de curva de fluxo pode ser atribuído a grande quantidade de fibras presentes na polpa, as quais promovem uma separação de fases na polpa quando esta é submetida ao cisalhamento

• A polpa de gabiroba apresentou comportamento viscoelástico com características de gel nas análises oscilatórias, destacando-se também pela sua estabilidade térmica, indicando que a polpa pode ser utilizada em processos que envolvam congelamento e aquecimento sem alterar suas propriedades reológicas, o que a torna interessante principalmente para a indústria de alimentos.

• A geleia de gabiroba formulada a partir da polpa, apresentou comportamento pseudoplástico descrito pelo modelo matemático de Herschel-Bulkley, estando de acordo com o comportamento observado na literatura para geleias de frutas.

• A geleia analisada apresentou-se estável, mantendo o comportamento reológico de gel no período de até 22 semanas de armazenamento, característica interessante que pode auxiliar no desenvolvimento deste produto para a comercialização.

De acordo com os resultados obtidos, podemos ressaltar as contribuições deste trabalho de doutorado principalmente para o conhecimento da espécie de gabiroba (*Campomanesia xanthocarpa* Berg), bem como da família Myrtaceae, para as quais os estudos encontrados na literatura ainda são superficiais do ponto de vista científico.

A análise da estrutura fina dos polissacarídeos extraídos da polpa de gabiroba, apresentada pela primeira vez na literatura através do presente trabalho, mostrou que essa fruta é uma fonte de pectinas com alto grau de metil-esterificação que podem ser aplicadas em diferentes áreas. Além disso, a presença de galactoglucomananas extraídas da polpa de gabiroba e caracterizadas, é inédita em frutos pertencentes a família Myrtaceae.

O estudo do comportamento reológico mostrou que a polpa de gabiroba é promissora como matéria prima para a aplicação em diferentes produtos, pois é capaz de manter suas propriedades reológicas principalmente quando submetida aos processos de congelamento ou aquecimento. Assim, a polpa poderia ser comercializada congelada para a produção de sucos por exemplo, ou ser utilizada em processos que envolvam seu aquecimento, como é o caso da fabricação de geleias.

A geleia de gabiroba formulada utilizando a polpa, além de apresentar um sabor diferenciado, mostrou-se estável em relação as suas propriedades reológicas, quando

analisada quanto ao tempo de armazenamento. Esta informação, juntamente com as outras características reológicas da geleia, pode ser grande aliada no desenvolvimento de uma geleia de gabiroba como produto para a comercialização.

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